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Culturing benthic foraminifera to understand the effects of changing seawater chemistry and temperature on foraminiferal shell chemistry

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Culturing benthic foraminifera to understand the effects of changing seawater chemistry and temperature on foraminiferal shell chemistry

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NOMENCLATURE

EMP	Electron microprobe
EMPA	Electron microprobe analysis
FAD	First appearance datum
HMC	High-magnesium calcite
IMC	Intermediate-magnesium calcite
LMC	Low-magnesium calcite
PAR	Photosynthetically active radiation
PVC	Poly-vinyl chloride
SST	Sea surface temperature

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ABSTRACT

The Cenozoic has been characterized by global cooling. However, the timing and magnitudes of this transition remain clouded due to conflicting evidence. The Mg/Ca of foraminiferal calcite has been shown to be an accurate proxy for paleo-seawater temperature, which when combined with the $\delta^{18}O$ of foraminiferal calcite, can be used to decipher the timing of glaciations by calculating the $\delta^{18}O$ of paleo-seawater. For this to work over long time scales (greater than 10^6 years), secular variation in seawater Mg/Ca must be accurately accounted for. Currently, there is a paucity of calibrated models that describe how Mg/Ca of foraminifera respond to variations of seawater Mg/Ca, despite extensive calibration of multiple species for temperature. Benthic foraminifera in particular, are one of the best proxies of bulk ocean properties, because they inhabit deep-ocean water masses less affected by high-frequency variations in sea surface temperature and salinity. To this end, a colony system was built to provide a constant supply of benthic foraminifera for experimentation. This culture system was designed after a thorough review of academic literature and in consultation with local aquaculture, engineering, and biology experts. This system currently houses live species of foraminifera from several locations around the world, such as Long Island Sound, Qatar, and the United Arab Emirates. To investigate how foraminiferal Mg/Ca responds to varying seawater Mg/Ca and temperature, 600 specimens of the high-Mg calcite foraminifer *Peneroplis planatus* were placed in experimental microcosms of varied seawater Mg/Ca and temperature. Of these 600 foraminifera, 102 survived the duration of the experiment and were analyzed via electron microprobe. These geochemical data show a positive, power law correlation between seawater Mg/Ca and foraminiferal Mg/Ca measured in the third chamber of growth at 25°C. This analysis supports a similar study done on high-Mg calcite foraminifer *Operculina ammonoides*. Geochemical data from specimens grown at other temperatures (3°C and 12.5°C) were inconclusive. Further research is needed to characterize the response of foraminiferal calcite to changing water Mg/Ca at seawater temperatures other than 25°C. This is necessary because the behavior of this proxy must be verified at temperatures that are similar to oceanic bottom waters (and thus benthic foraminiferal habitats).

CHAPTER I. INTRODUCTION

1.1 Background

Foraminifera are an invaluable asset to paleoclimatologists, ecologists, biologists, paleontologists, and oceanographers because their shell chemistry records aspects of the environment in which they grew. For example, researchers have been able to decode the following parameters from foraminiferal shell chemistry: sea surface and deep-sea temperatures (Rosenthal et al., 1997; Kucera et al., 2005), extent of continental glaciation (Lear et al., 2000; Zachos et al., 2001), ocean oxygenation (den Dulk et al., 1998), and carbon cycling (Mackensen and Bickert, 1999). They can be used in biostratigraphy (Heinrich, 1988), paleobathymetry (Eicher, 1969), and reconstruction of depositional events, such as turbidity current flow and deposition (Piper and Savoye, 1993). In addition, they can be used to assess environmental pollution and remediation progress (Morvan et al., 2004).

As widely used as foraminifera are, it is still not fully understood how environmental parameters become encoded in their shell chemistry (Keul et al., 2013, Pawlowski et al., 2013). Culture studies are a logical way to test the response of shell chemistry response to environmental variables and because the culture environment can be more easily controlled. Reports of foraminiferal culturing date back into the 19th century (e.g. Gervais, 1847; Schultze, 1856; Myers, 1937). These studies largely focused on foraminiferal biology, especially reproduction. More recent culturing endeavors have focused on calibrating paleoenvironmental proxies based on test chemistry (Delaney et al., 1985; Nurnberg et al., 1996; Hintz et al., 2004; Bentov and Erez, 2006; Vetter et al., 2014; Spero, 2015). The paucity of these studies is largely due to the difficulty in culturing foraminifera that often arise when trying to maintain sterility or mimicking natural environments in laboratory microcosms.

Test mineralogy and trace element chemistry vary from taxonomic order to taxonomic order and are much more complex than originally thought (Pawlowski et al., 2013). In addition, recent recognition of heterogeneity in test chemistry has made analysis more difficult (Toyofuku et al., 2011). Though all foraminifera are heterotrophic, each species has its preferred diet (Lee, 2010). In order to maintain foraminifera in long-term cultures, it is necessary to understand the biology, ecology, and behavior of the particular foraminifer being cultured, as well as its food source. The environmental niche of a cultured foraminifer must be mimicked in the lab as close as possible. Deep-water benthic foraminifera, such as agglutinated varieties, are especially difficult to culture because of the complicated experimental setup needed to maintain foraminifera at deep ocean pressures (Hemleben et al., 1990). In addition to recreating their natural environment in a culturing system, food cultures must also be maintained to provide the foraminifera with a diet that most closely matches their preference.

Most culture studies with foraminifera rely on acquiring specimens from the ocean and immediately introducing them into experimental conditions (Bijma et al., 1990; Spero and Lea, 1993; Sanyal et al., 1996). This method is not always practical for scientists living far from the ocean (e.g. Iowa).

1.2 This Study

For this project, the overarching research goal was to understand how foraminiferal test Mg/Ca varies with seawater Mg/Ca and seawater temperature (T). In a broader context, test Mg/Ca has been proposed as a proxy for paleo-seawater temperature (Rosenthal et al., 1997). However, calcite Mg/Ca also varies with seawater Mg/Ca (Ries, 2004) and seawater Mg.Ca has varied significantly over the Phanerozoic with changes in seafloor spreading rates (Stanley and Hardie, 1999). The residence time of Mg and Ca is on the order of millions of years—much longer than the time required for the ocean to effectively mix (Stanley et al., 1999). Therefore, these two parameters can be integrated into a single transform function describing how calcite Mg/Ca varies as a function of both seawater temperature and Mg/Ca (Ries et al., 2004; Hasiuk and Lohmann, 2010; Nurnberg, 1996). By inputting Cenozoic trends in foraminiferal $\delta^{18}O$ (Zachos et al., 2001), foraminiferal shell Mg/Ca and seawater Mg/Ca (Hardie, 1996) into the appropriate transform function derived from experimentation, we can calculate paleo-seawater $\delta^{18}O$, a proxy for continental ice volume (Lear et al., 2000). Past attempts at deciphering the transition over the Cenozoic from greenhouse climate to the late Cenozoic icehouse climate have disagreed over the timing and extents of continental glaciation, with most favoring Northern Hemisphere glaciation only in the Pleistocene (Lisiecki and Raymo, 2005; Zachos et al., 2001). Others have identified signs of Northern Hemisphere glaciation as far back as the Eocene (e.g. Tripati et al., 2008) based on the presence of ice rafted debris in marine sediments. Culturing foraminifera allows these conflicting hypotheses to be tested, with respect to timing and magnitude of global continental ice volume accumulation, though no distinction between northern or southern hemisphere glaciation can be determined.

1.3 Previous Research

Previous culture studies have been done on foraminifera, but these introduced specimens directly to experimental conditions and none were kept aside as a base supply (Chandler et al., 1996; Wilson-Finelli et al., 1998; Havach et al., 2001; Hintz et al., 2004). Systems to maintain foraminferal cultures long-term (months to years) have not been described recently in the literature (e.g. Zillioux, 1969; Chandler et al., 1996). Such a “colony” can provide a continuous supply of test subjects for culture experiments. The objective of the first half of the study was to build a benthic foraminifera culture system that can be located geographically anywhere to provide a source of healthy specimens for experimentation. The objective of the second half of this study was to use these colony residents as test subjects in experiments that determine the relationship between the Mg/Ca and T of the seawater and the Mg/Ca in a foraminiferal test.

1.4 Oxygen Isotope Fractionation

$\delta^{18}O$ varies with continental glaciations due to the fractionation of oxygen isotopes during times of climate change. Water evaporates from the ocean and both ^{18}O and ^{16}O are taken up. However, ^{16}O is preferentially removed due to its lighter weight. As water vapor moves inland and loses mass due to precipitation (which is preferentially enriched in the heavier isotope ^{18}O), more ^{18}O is returned to the seawater, further increasing the ratio of $^{18}O/^{16}O$ in seawater. During times of glaciation, the ^{16}O that was preferentially removed by the evaporative process is locked up in continental ice and not returned to the ocean. During warmer periods with fewer/no glaciers, the ^{16}O-rich water is returned to the ocean. This leads to oceans with more positive $\delta^{18}O$ during times of continental glaciation, and oceans with more negative $\delta^{18}O$ during times without continental glaciation (Lisiecki and Raymo, 2005). Foraminifera trap this same fractionation in their test, essentially recording the contemporaneous seawater $\delta^{18}O$ (Lisiecki and Raymo, 2005). During the Cenozoic, benthic foraminifer $\delta^{18}O$ has been shown to range over 5.4‰, with values as high as 0.2‰ at the Paleocene-Eocene thermal maximum and as low as -5.2‰ today (Zachos et al., 2001). These estimates were derived from Ocean Drilling Project cores. Though $\delta^{18}O$ varies with salinity and source water contributions, the major contributor to fractionation in foraminiferal tests is due to this change in global ice volume (Lisiecki and Raymo, 2005; Zachos et al., 2001).

Though foraminifera have existed since the Cambrian, special attention must be given to selection of species when concerning culture studies for paleo-environmental and paleo-cryospheric analysis. Foraminiferal orders are determined by metabolism (Pawlowski et al., 2013). Extant foraminifera should be from the same order as the foraminifera from the rock record being used for analysis; the same species is not necessary due to similarities in biocalcification within a taxonomic order (Pawlowski et al., 2013). Additionally, the foraminiferal order needs to have existed (have its first appearance datum) at, or prior to, the time period in question. If possible, it is best to choose a taxon that has existed since the start of the geological time period of concern due to evolution within foraminiferal orders and possible metabolic changes during genetic divergence (BouDagher-Fadel, 2008).

1.5 Foraminifera Experiments

For initial experiments, two species were used: *Peneroplis planatus* and *Elphidium excavatum*. *P. planatus* was the foraminifera used for the most comprehensive and successful study, and the only study for which geochemical analysis was conducted. *Peneroplis* makes a good test subject because of its relatively large size, which makes them very easy to work with. All of our specimens were approximately 1 mm in diameter. Additionally, the time period of concern was the Cenozoic Era, particularly the last 50 million years. Peneroplids evolved approximately 55 Ma, at the Paleocene-Eocene boundary (BouDagher-Fadel, 2008). Modern

Peneroplis planatus occupies shallow, intertidal marine depositional environments and are not adapted to deeper pressures or low light conditions. They have endosymbiotic algae that are common and easy to grow in culture (Sen Gupta, 1999). This species is also very hardy due to the variable conditions they experience in their natural environment (e.g. changing salinity, light, vegetation, and water depth).

1.6 Thesis Outline

The main body of this thesis is composed of two papers. Chapter II, the first of the two papers, describes the foraminiferal culture system in which various species of benthic foraminifera were grown. This new system is compared to two previously reported culture systems: one designed by Hintz and colleagues (Hintz et al., 2004), and the other by Ries (Ries, 2004; Ries, 2006; Ries et al., 2006; Ries, 2010). Chapter III discusses experiments done with *Peneroplis planatus*, and the resulting transfer function obtained which predicts seawater Mg/Ca based on foraminiferal Mg/Ca for peneroplids at 25°C. This function is likely applicable to the other species in the genus *Peneroplis* because they metabolize similarly. This function can probably also be applied to other large benthic foraminifers that produce high-Mg calcite tests and live in warm waters. This function is likely not applicable to taxonomic order other than *Peneroplis,* and cannot be used if the deep-sea temperature differed from 25°C.

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CHAPTER II. A RECIRCULATING SYSTEM TO SUPPORT A COLONY OF BENTHIC FORAMINIFERA

A manuscript to be submitted to Limnology and Oceanography Methods

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2.1 Abstract

A recirculating system was designed and built to provide a constant supply of foraminifera for experimentation. Though some designs exist in literature, they were not suitable for long-term culturing of foraminifera. This culture system was designed after a thorough review of the literature and in consultation with local aquaculture, engineering, and biology experts. It is composed of a central seawater reservoir that feeds several independent 6 L culture chambers. Water returns to the seawater reservoir by overflowing the culture chambers and passing through a multistage physical, chemical and biological filtration system. This system currently houses live species of foraminifera from several locations around the world, such as Long Island Sound, Qatar, and the United Arab Emirates. This system can easily be expanded or downsized to meet the needs of the researcher. It is composed of common parts, built for less than $2,400, and costs less than $840 per year to maintain. Having access to a low-cost system allows researchers far from the ocean to have a continuous supply of foraminifera for experimentation, thus removing locational barriers for research.

2.2 Introduction

Typically, foraminiferal culture experiments are conducted over short terms (less than three months), with test subjects being replenished from natural habitats in the ocean for each experiment. Currently, only a handful of foraminiferal culture systems have been described in literature (e.g. Zillioux, 1969; Chandler and Green, 1996). However, in order to conduct multiple culturing studies far from a source of new test subjects, a longer-term system is needed to maintain active foraminiferal cultures. The objective of this study was to design and construct a culture system capable of supporting foraminiferal cultures indefinitely that could provide specimens for various experiments. These experiments seek to measure the relationship between foraminiferal Mg/Ca and seawater Mg/Ca and Temperature.

Two other systems have been well documented, one for the culturing of benthic macro-invertebrates (Ries, 2004; Ries, 2006; Ries et al., 2006; Ries, 2010) and another for benthic foraminifera (Hintz et al., 2004). Both systems designed with the goal of subjecting test specimens to artificial seawater with varying trace element ratios at constant temperature.

Ries (2004) evaluated how carbonate secreting organisms respond to changing seawater Mg/Ca. Mg/Ca has been suggested to be a major driver the observed oscillations in the abundance of aragonite vs. calcite secreting organisms over the Phanerozoic (Stanley and Hardie,

1999). The end objective was to evaluate how seawater Mg/Ca affects skeletal Mg/Ca of these organisms in order to test whether marine calcifiers would secrete lower-Mg calcite shells in seawater of lower Mg/Ca (Ries, 2004). Four species were used: serpulid worms (*Hydroides crucigera*), crabs (*Perchon gibbesi*), echinoids (*Eucidaris tribuloides*), and shrimps (*Palaemonetes pugio*) (Ries, 2004). Six organism of each species were housed together for 160 days in experimental seawater. This seawater was made by Ries using Kester et al.'s formula in Bidwell and Spotte (1985). This system utilized 10-hour light/14-hour dark cycle. No substrate was used, and the system was kept at 25°C. The high survival rate of the test subjects suggested that Mg/Ca does not strongly affect their metabolism or vitality. Chemical analysis of experimental calcite was done via electron microprobe analysis (EMPA). No other specifics on system design, operation, maintenance or theory were reported.

Hintz et al. (2004) cultured benthic foraminifera to calibrate paleo-proxies of ocean productivity (Ba/Ca and Cd/Ca). The 1600-liter, recirculating system had an adjustable number of culture chambers. This system was kept between 6.6 and 8.7°C. The Hintz system used silica substrate and was a longer-term system than the typical 90-day cultures, in that it was used for greater than 200 days. Foraminifera were shown to have reproduced in this system. Reproduction was certain because chambers were sealed at experiment initiation with 30-50 adult foraminifera in each chamber, and hundreds of juveniles were present upon completion of experiments. The Hintz system was well documented and thus derivatives of it have been employed by numerous others (e.g. Raitzsch et al., 2010; Duenas et al., 2011; Allison et al., 2011).

The plastics used were chemically inert Teflon, polyethylene, and polypropylene (Hintz et al., 2004). Seawater was kept at a salinity of 35‰ and was formulated from a commercially available seawater mix (Instant Ocean). Ba was removed and Cd was added to create conditions closer to their specific site of interest. The culture chambers for this system were custom milled in two halves and that sealed with an O-ring. The culture chamber volume was 19 mL. Each culture chamber had an air stone to provide oxygen. It was not left open to room air, though water continually flowed into and out of the chambers. Foraminifera were fed algae weekly through a small port at the top of the chamber.

Being located in central North America, circa 1,600 km from the ocean, it would prove difficult for an Iowa-based oceanographer to collect foraminifera from the ocean on a regular basis for culture studies. Additionally, invertebrates incur stress when transplanted from a native environment into a laboratory setting (Spees et al., 2002). This stress translates into slowed growth, disrupted metabolism, and even fatality (Spees et al., 2002). Having foraminifera that are not stressed at the time of collection, as might be expected when collected directly from a continuous laboratory culture, has been suggested to yield better outcomes in experimental studies (Spees et al., 2002). An inexpensive foraminiferal colony system was designed that easily maintained multiple, independent cultures far from a supply of natural seawater, without cross contamination. This low maintenance system can be easily expanded or reduced to fit the researchers needs. Unlike the Hintz system, this system has a large central filtration system,

easily accessible colony reservoirs, is made from readily available and inexpensive building supplies, and has maintained a foraminiferal colony for over a year. We also propose using natural substrate collected along with foraminifera to produce a less stressful culturing environment.

2.3 Methods

2.3.1 Component One: Laboratory

The system occupies approximately 6 m^2 of lab space. Materials were purchased from various retailers. See Table 1 for a list of all supplies used, product numbers, and purchase location. The system has four major components: the laboratory, the seawater reservoir, the colony reservoirs, and the filtration subsystem (Figure 1) as well as plumbing to connect them all together. In addition, proper illumination is critical in maintaining sufficient algae growth to provide nutrition for the foraminifera. Care was taken to minimize potential contaminants (like leaching from plastics and metals). However, contamination is near impossible to avoid because substrate, Instant Ocean Seawater Mix, and even the laboratory air contain bacteria and other contaminants. This is much like the natural ocean where foraminifera originate.

This culture system was housed in the basement of a classroom and laboratory building (Science Hall) on the campus of Iowa State University in Ames, Iowa, USA. The lab was equipped with a non-opening window. Temperature was maintained by balancing a window air conditioner and a wall-mounted radiator. Ventilation was provided by the window air conditioner and by a fume hood located in the room. Temperature was kept at approximately 25°C. Temperature never exceeded 26°C and never dropped below 24.5°C. Pursuant to North American building codes, outlets within 1.2 m of a water source were protected by a ground fault circuit interrupter (GFCI). The countertop holding the colony was waterproofed using an epoxy paint.

2.3.2 Component 2: Seawater Reservoir

A 568 L tank was purchased to use as the seawater reservoir. This volume is approximately 20 times larger than the total volume of the colony reservoirs, providing a chemical buffer against biomineralization affecting seawater chemistry in the system. After installation, it was cleaned using dish soap, rinsed several times in tap water, and finally, rinsed three times with deionized water. The seawater reservoir was made of chemically inert plastic (polyethylene) that is commonly used in industrial-scale aquaculture. A 1-cm-thick lexane lid was fabricated to cover the entire reservoir, leaving spaces for tubes to enter and exit. A small submersible pump (1,250 liter per hour) ensured vigorous mixing within the seawater reservoir. Plastics used in pump construction were created specifically for use in aquatic habitats.

2.3.3 Component Three: Colony Reservoirs

Cubic, clear, polycarbonate, 7.5 liter containers were used for the colony reservoirs. Each colony reservoir was modified before integration with the larger system. A 2.5 cm hole was drilled in the lid of each container to allow water inflow. A 1.9 cm hole was drilled in the back of the container, near the top, to accommodate a 40 cm outflow hose that connected to a common drain pipe, leading back to the filtration subsystem. A barbed, nylon bulkhead union allowed the outflow hose to attach directly to the back of each colony reservoir. While the system constructed in this study included seven individual colony reservoirs, more reservoirs can be added to the system (or fewer reservoirs used) without substantial modification to the overall design.

2.3.4 Component Four: Plumbing

The system was designed with a single pump supplying seawater from the seawater reservoir to the colony reservoirs. Wastewater overflowed from the colony reservoirs and drained by gravity into the filtration subsystem, filtered water from which overflowed and drained by gravity back into the seawater reservoir.

2.3.4.1 Supply Plumbing

A larger pond pump (7,570 liter per hour) supplied artificial seawater to the colony reservoirs. Metal hose clamps were used to attach tubing to pumps and also to attach the larger pump to the main supply pipe. No air stones were employed to oxygenate the seawater reservoir. The trickling of water through the system, and the cascading of water from the filtration to the seawater reservoir provided sufficient oxygenation. Sufficient oxygenation was assumed due to healthy macro-algae growth (Figure 2) and normal pH (8.3), because low pH is correlated with low oxygen levels. Plastics used for the pumps were created specifically for use in aquatic habitats, so it was assumed that there was minimal leaching from the pump parts.

One-inch, flexible tubing barb with a hose clamp was used to attach the supply tubing to the supply pump in the seawater reservoir. After the distribution pipes were plumbed for each colony reservoir, they were hung from overhead cabinetry using plumbing straps. The distribution plumbing was constructed using 1.27 cm polyvinyl chloride (PVC) pipe and stop valves with Tee connectors. At the end of the branch to each colony reservoir, flexible, 1.27 cm, silicone tubing was used to allow more flexibility with colony reservoir placement on the counter. Each piece of silicone tubing was approximately 40 cm long. Each connection was secured with a hose clamp.

2.3.4.2 Drainage Plumbing

The drainage plumbing consisted of 3.18 cm PVC pipe plumbed with 1.9-1.27 cm Tee connectors. A cap was placed on the end of the pipe and a 3.18-3.18 cm Tee connector coupled another 3.18 cm pipe to drain to the filtration subsystem. A pipe was also run to a sink to allow complete system drainage. A return cycling branch was constructed, with its own stop-waste

valves. The return cycling branch and a portion of the drainage branch were also secured to the counter using plumbing straps.

2.3.5 Component Five: Filtration Subsystem

The filtration subsystem (Figure 5) was constructed using a 75.5 L plastic tank. This tank was composed of the same chemically inert, UV-stabilized, polyethylene that composed the seawater reservoir. A lexane lid covered the filtration subsystem, with holes to allow water inflow from colony reservoir discharge. Lexane dividers were installed in the tank to separate the mechanical/chemical section of the subsystem from the biological filtration. Dividers were glued in place using clear silicone sealant in a fume hood.

Mechanical and chemical filtration in the filtration subsystem consisted of several layers (Figure 5). The first stage of filtration involved the mechanical removal of suspended solids using successively smaller mesh filter media. Below that, a layer of a layer of activated carbon pellets in mesh bags, wrapped in filter fabric, acted as a chemical filtration. This aided removal of heavy metals, chlorine, and nitrates. The bottom layer consisted of zeolites. For extra biological filtration, *Chaetomorpha* was utilized in the central portion of the filtration subsystem. A 30.5 cm refugium grow light provided the appropriate illumination for *Chaetomorpha* growth. It was placed on top of the central portion of the filtration subsystem. Holes were drilled on the short side of the filtration tank to discharge filtered water into the seawater reservoir by gravity. Nylon, barbed bulkheads were chosen from available options to attach the discharge tubes, as it does not leach chemicals.

2.3.6 Component Six: Illumination

Some species of foraminifera host endosymbionts that provide the foraminifer with nutrition (e.g. Hallock and Peebles, 1993; Pawlowski et al., 2001). In some cases these endosymbionts are required for foraminifera to maintain a healthy metabolism (Pawlowski et al., 2001). The illumination for this system was designed to promote the growth of the foraminiferal endosymbiont, *Porphyridium*. "Red algae" growth spectrum lights (60 cm long) rested on the top of each colony reservoir on a 12-hour light/dark cycle (Figure 3). A 60° beam angle was selected to concentrate as much of the light on the bottom of each colony reservoir at the desired photosynthetically active radiation (PAR) levels (Figure 4). This choice was based on manufacturer's recommendation. PAR can also be called photosynthetic photon flux and is a measure of the usable radiation. PAR is lost with water depth.

This colony design housed foraminifera that were under approximately six inches of water (15 cm). There was 10.8% absorption loss and the radiometric spectrum remained close to that of the radiometric spectrum in open air. If foraminifera are placed under a thicker column of water, more absorption occurs as does loss of red, orange, yellow, and green spectrum light. Photosynthetic photon flux density is a measure of what PPF reaches the organism through a

medium (water). PPF is attenuated both by the oragnisms' distance from the center of the fixture, as well as water depth (Figure 4D).

2.3.7 Nutritional Algae Culture System

To feed the foraminifera, a separate nutritional algae culture system was designed and built (Figure 6). Algae were purchased and cultured according to vendor's instructions. A wood frame was constructed to hold a 90-cm light ("red algae" spectrum, 60° beam angle) over the algae reservoirs (Figure 7). The light was fixed to the frame and set on a 12-hour light/dark cycle using a generic appliance timer. An air splitter and pump oxygenated algae reservoirs (Figure 6).

Algae cultures required weekly maintenance. First, if any water had evaporated deionized water was added to get each culture back to normal salinity (35‰). Next, half of the culture was discarded. Then, two drops of "F2" culture medium were added to the algae reservoirs and artificial seawater (Instant Ocean mixed with deionized water) was added to fill each bottle back to 1 L (Guillard and Ryther, 1962). The algae culture bottle was sealed and the air tube re-inserted.

2.3.8 Seawater Recipe

Instant Ocean Seawater mix and deionized water were mixed to create artificial seawater for use in the foraminiferal culture system. Instant Ocean Seawater mix most closely resembles natural seawater in chemistry and salinity (Atkinson and Bingham, 1997). The system was circulated for approximately one month before introducing foraminifera. This circulation promoted cleaning of the plastic and assured integrity of plumbing connections. While running, the sink discharge valve was closed and all other valves were open. Flow was set using colony reservoir inflow valves to a rate that allowed at least 60 liters per hour to flow into each colony reservoir.

Water changes were done to remove nitrates, accumulated waste, phosphates, and excess algae in the system. During water changes in the seawater reservoir, the valve to the sink was opened first. Then the main valve to the individual colony reservoirs was closed. The pump was left on to circulate the seawater reservoir. One third of the water was drained to the sink and then the valve to the sink was closed. New water and Instant Ocean mix was added to the seawater reservoir to replace the removed water. After salinity tests read 35‰, the seawater reservoir was left to circulate for half an hour and retested to assure proper salinity. Once desired salinity was reached, the main valve to the individual colony reservoirs was opened.

2.4 System Commissioning and Maintenance

Aquaculture-grade chemistry test strips were used to monitor pH, alkalinity, nitrates, nitrites, hardness, and chlorine. If any parameter was outside of the ideal conditions (as noted on the test strip kit), a one-third water change was done on the seawater reservoir. If water was not changed one week due to ideal water conditions, a one-third water change was done the

following week, regardless of whether the parameters were within desired limits. The coarse filter media in the filtration subsystem components was rinsed with tap water during every water change and completely replaced monthly. The fine media was changed every other week and the carbon was changed with every water change. After one month, the carbon did not generally provide adequate chemical filtration, according to the manufacturer, so its use was discontinued after a month.

2.5 Results

2.5.1 Introduction of Foraminifera

Foraminifera were collected from several locations around the world (Figure 8) and shipped back to the colony by air. Foraminifera were grouped in colony reservoirs by location rather than by species. Natural substrate, collected *in situ* with the foraminifera, was used to provide foraminifera in an environment as close as possible to their own natural environment. During introduction to the system, foraminifera were acclimated over the course of an hour where artificial seawater was added to their travel container until it was approximately 75% artificial. After acclimation, system circulation was deactivated and sediment and foraminifera from a single location were placed in a single colony reservoir. System circulation remained off for an hour to allow foraminifera, algae, other organisms and sediment to settle to the bottom.

Several groups of foraminifera are currently housed in the system (Figure 8). The first foraminifera added were from Al Thakira, Qatar (shallow intertidal zone at 25° 45' 7.01" N and 51° 33' 50.12" E), in January of 2014. This group consisted mainly of *Peneroplid* species, though some smaller milliolid and rotalid species were present in small numbers. The peneroplids took especially well to the culture system as evidenced by their growing numbers and activity of climbing up hair algae that grew in the colony reservoir.

The second group of foraminifera was collected and added in March of 2014. This group came from Punta Cana, Dominican Republic (18° 43' 35.01" N, 68° 27' 30.05" W), from a shallow (approximately 1 m), intertidal environment. This group consisted of many different taxonomic orders of foraminifera, including Rotalida, Milliolida, and Buliminida. All types of foraminifera survived, however, the rotalids showed the most activity (movement across the substrate). They also consumed algae more often than the *Milliolida and Buliminida* foraminifera, as evidenced by their brighter colored cytoplasm.

The third group, added in August 2014, came from a subtidal offshore marine environment off of the coast of Scotland in 76 meters of water (57° 3.447’ N, 6° 28.291’ W). This assemblage resembled foraminifera from the Dominican Republic, with respect to genus. However, they were much smaller in size. This is likely due to the greater collection depth. They did not appear to eat much of the food or move around a lot. They seemed to still be alive when the colony reservoir was cleaned out (January 2015, six months after introduction), but they did not appear as healthy as the rest of the foraminifera in the culture system, with some being bleached completely.

The next specimens added to the system were collected at Joshua Cove (an erosional environment) in Long Island Sound, off of Connecticut, USA (41° 15' 3.78" N, 72° 43' 31.31" W in approximately 20 meters of water). This group consisted mainly of *Elphidium excavatum*. There were two morphologies present. Most of the foraminifera arrived dead. Live foraminifera were picked out in large numbers and added to the system. They were alive at the time that the colony reservoir was emptied on (June 2015, five months after introduction), as evidenced by their bright cytoplasm.

The final group of foraminifera that were added to the system was collected from a shallow intertidal zone at Kite Beach, Abu Dhabi, United Arab Emirates (24° 30' 48.93" N, 54° 32' 54.42" E, February 2015). All foraminifera collected from UAE were peneroplids. A majority of them were very large in size (2 mm in diameter or larger) and easily identifiable among the sediments they were collected with. Within one day of being placed in the system, they consumed algae and moved around their colony reservoir. These foraminifera had very brightly colored cytoplasm upon examination under stereomicroscope.

2.5.2 Status of Foraminifera Cultures

This system has maintained live foraminiferal cultures for approximately a year and a half. It is unclear if they have reproduced while in culture (no calcein was added to the system initially, and foraminifera were not counted). Calcein is shown to stress foraminifera, so adding it to the entire system is not advised (Kurtarkar et al., 2015). Reproduction was likely not impeded by poor water conditions because conditions always tested in the ideal range. The foraminifera also appeared to be consuming algae that were added to the system.

Seven colony reservoirs have housed foraminifera from the four aforementioned locations. No organisms, including algae or foraminifera, appear to have transferred between colony reservoirs. This suggests that the filtration subsystem is effective at cleaning the discharge water. Since significantly different algae grow in the different colony reservoirs, it is clear that algae do not migrate between the colony reservoirs (Figure 2).

2.5.3 Feeding of Foraminifera

Foraminifera in the culture system were fed every two weeks with the mixture of *Porphyridium* and various species of pennate and centric diatoms. To feed foraminifera, water flowing into the colony reservoirs was deactivated for approximately an hour to allow time for algae to settle to the bottom. Approximately 10 mL was taken from a concentrated area of algae cultures (prior to weekly splitting and addition of F2 media) and put in each colony reservoir. After an hour, the system was re-activated.

2.6 Discussion

2.6.1 Comparison to Previous Systems

The system described in this study differs fundamentally from those often described recently in the literature. This colony system aims to recreate the natural ecosystem more accurately than the sterile conditions of Hintz et al. (2004) or Ries (2004). By introducing foraminifera *and* co-occurring sediment (with all the associated microflora and microfauna) it is hoped that a more natural ecosystem might be created and maintained in the lab, an ecosystem that might be more likely to survive long-term so far from its natural environment. Due to the fact that colony inhabitants are not being experimented on directly while they are in the colony, the system can be designed to for optimal long-term foraminiferal growth.

Similar to our system, Ries used artificial seawater that was lab-created (mixed from component salts and chemicals) to house calcite secreting organisms (Ries, 2004). For his experiments, as with our experiments (cf. Chapter III), the artificial seawater formula was taken from Kester et al.'s formula in Bidwell and Spotte, (1985). Both our colony system and the Ries experimental system were kept at 25°C (Ries, 2004).

There were several differences between our system and the system designed by Ries. The major difference is that the Ries system was only used for short-term, experimental studies (Ries, 2004). This was not a long-term system that was created to facilitate a stock of organisms or organismal reproduction (Ries, 2004). Ries housed all species separately while in the system. In contrast, in our colony system taxa were housed together by location of collection to help maintain the original ecosystem as close to natural as possible. Another major difference is that Ries did not use substrate (Ries, 2004). Certain species of foraminifera are infaunal, and without this substrate, their metabolism could be severely disrupted. Therefore, substrate is necessary when culturing and experimenting with infaunal species (Murray, 1991).

These differences led us to design a significantly different system for maintaining our foraminiferal colony. Although the Ries system was used for an extended time, 160 days, it was not long enough for the purposes we needed it for (indefinitely). To accommodate substrate in the design of the colony, as well as multiple organisms and microorganisms, a different setup is needed that includes a larger filtration system in order to keep cyanobacteria, diatoms, etc. from blooming and consequently lowering the pH of the system and consuming available oxygen. Additionally, only a few details, design, or maintenance of the Ries experimental system were reported in publication (and these details did not include parameters such as number of colony reservoirs, filtration system, buffering of the water, etc.), so it would be difficult to create that system on our own.

The Hintz system (Hintz et al., 2004) has several similarities to the colony system designed in this study system. Both systems were specifically designed for benthic foraminifera in a way to get them to reproduce in captivity, though our colony system was not being used for experimentation. Both systems had an adjustable number of reservoirs and used similar plastics for construction. Like our system, the Hintz system is a long-term system with a goal of

foraminiferal reproduction. Both the Hintz system and our system used a large seawater reservoir to help buffer the water from any changes in chemistry caused by foraminiferal biomineralization, and both used Instant Ocean seawater mix to create seawater.

Unlike our system, the Hintz system is not as easy to build or nor does it afford easy access to foraminifera. Chambers were custom built, and not something that can be purchased pre-made. Each chamber was milled in two halves and sealed together, with an air stone in each chamber to provide oxygen (Hintz et al., 2004). Foraminifera were fed weekly. A similar schedule was used initially in this study, though it was found to be excessive because diatoms and other algae reproduced on their own within our system in large enough amounts to provide sufficient nutrition for the colony foraminifera to remain healthy (colored foraminiferal cytoplasm suggested they were eating these algae). Each reservoir of the Hintz system was only 19 mL (0.019 L). This is in large contrast with our multiple 6-liter colony reservoirs. Silica substrate was used in the Hintz system. Silica was specifically avoided in this study because of the potential for diatom blooms occurring due to the presence of excess dissolved silica (Nelson et al., 1995). Substrate in this study was composed of naturally occurring sediments collected with the foraminifera so the colony reservoir they were placed in would mimic their natural environment as closely as possible. Sediment was composed mostly of carbonate grains (Abu Dhabi, Dominican Republic, and Qatar samples) or clay minerals (Scotland sample), sometimes with biotite flakes (Long Island Sound sample). This did not cause issues with seawater carbonate chemistry. Over the course of this study, the colony system was never outside of "ideal" parameters: nitrates and nitrites were always at 0 ppm, total alkalinity was always between 180 and 300 ppm, and pH was always at 8.4 ±1.0. It is possible that the carbonate substrate helped buffer the system.

Though the Hintz system was specifically designed for benthic foraminifera, it was not suitable for the purposes of this study because its foraminifera are not easily accessible. It is necessary to extract foraminifera frequently to observe, identify, and separate them for experimentation. An open-air system was decided as an optimal design to facilitate such frequent intrusion. Easily removable, "snap-on" lids were used on each colony reservoir to minimize evaporation.

Because the presence of other algae, nematodes, etc. would kill the foraminifera over time, a larger filtration system is needed to remove these troublesome taxa as they reproduced. A separate filtration subsystem was designed in our system to have mechanical, biological, and chemical filtration to keep diatom and other algae from out-competing the foraminifera that were the focus of our study. Additionally, Hintz chambers were too small for our purposes. Peneroplids, an epifaunal species that prefers to climb on hair algae, were ultimately chosen as species of interest to culture. It would be difficult for a fair amount of hair algae to grow in chambers as small as those reported by Hintz (2004). Finally, the Hintz system was not ideal because several of the foraminifera had endosymbionts that needed a specific spectrum of light

to photosynthesize. A Hintz chamber would likely not allow that light to penetrate the chamber and reach the foraminifera.

2.6.2 Use of the System

The colony system described in this study is useful for a variety of different applications at different scales. Any number of colony reservoirs, of any size, can be integrated into the system. The total volume of the system should not exceed approximately 1100 liters. Anything from algae to vertebrates can be grown in this system. A similar multi-tank "overflow system" was observed at a local animal retailer, which had one approximately 25,000 liter (6,600 gallon) tank housing a *Mustelus canis* specimen (Dog Shark). All other small (approximately 30 8-liter tanks) saltwater tanks were also attached to this recirculating overflow system. However, this system, like many reported in the literature (cf. Timmons and Losordo, 1994; Heguenin and Colt, 2002), were optimized for growing large invertebrates that require significantly higher flow rates and more substantial filtration subsystems to handle the larger waste loads.

This system is also customizable to the researcher's needs. The number of colony reservoirs is easily expandable to house many different species or assemblages. Several taxa can be kept separate or together, and new taxa can be added to other colony reservoirs without interfering with currently established colonies. Similar to the system designed by Hintz et al. (2004), this system can also have fewer colony reservoirs to reduce maintenance time and the cost of consumables. Like the system designed by Ries, several kinds of organisms can be grown in this system, from algae to larger invertebrates like echinoids (Ries, 2004). Several cerithid snails survived collection in Qatar and lived in excess of a year in the colony system, as did two unidentified species of microscopic shrimp. Vertebrates can be housed easily in this system, provided that colony reservoir outlets are covered with a mesh to prevent nektonic organisms from exiting colony reservoirs to the filtration subsystem. To house corals, the system would need to be modified by adding actinic lights. Such lights provide certain wavelengths of blue light that corals require for optimal growth. Many corals contain cyan fluorescent proteins that require this low light intensity in the blue spectrum (D'Angelo et al., 2008). These spectra are absent in most commercially available aquaria lighting. In addition, these actinic lights must be raised above the colony reservoir tops because they emit enough heat to melt softer plastics if they rest on the plastic.

Multiple taxa may be housed in the same colony reservoir, though care should be taken that they do not negatively interact with each other (e.g. predation). Though Hintz created a system for benthic foraminifera, it may not be viable for time periods longer than their experiments (200 days). This is due to the fact that, at the end of the experiment, nematodes were found throughout the entire system (Hintz et al., 2004). This occurred because there was no filtration subsystem to remove them. Lack of filtration would also have allowed algae to grow unabated in the Hintz system, as contamination is almost always inevitable. Also, organisms that require a colony reservoir larger than 19 mL would not be suited for the Hintz system. The

system designed by Ries was likely not suitable for foraminifera, as it housed larger marine invertebrates, such as serpulid worms, crabs, echinoids, and shrimps (Ries, 2004). Systems that house larger organisms typically have higher water flow rates and a filtration type that is not an overflow type. This would create too strong of a current for foraminifera, hair algae, and the fine substrate that some foraminifera prefer. This system is customizable in colony reservoir size and shape as well, which allows for greater flexibility in building a system that suited for a particular taxon.

Other systems that have grown foraminifera for the short term would also not work for our purposes due to inaccessibility and lack of long-term components (i.e. filtration subsystem to filter out algae, nematodes, etc.). A system was designed by Keul et al. (2013); however, it used petri dishes to house foraminifera in airtight boxes. This would not allow easy access to foraminifera or a natural environment for long-term foraminiferal growth. It is difficult to use substrate with foraminifera grown in petri dishes because other organisms will outcompete foraminifera if they are not filtered out (e.g. diatoms, ciliates, nematodes, etc.). A system similar to the Keul system, designed by Diz et al. (2015), also lacked the filtration subsystem of sufficient size to remove ciliates, which prevented foraminifera from reproducing. This new system design has the filtration and open-air access that these other systems lack.

This system is also very different from well know planktic foraminifera culture techniques. These techniques typically involve culturing in small containers (Spero et al., 1993; Spero et al., 2015). Foraminifera are collected from the ocean, sieved and immediately placed in experimental conditions (often in jars or petri dishes) for short amounts of time (approximately 90 days, Spero et al., 1993; Spero et al., 2015). Again, these planktic culturing methods lack open-air access and a large central filtration system that would make a system viable for longer-term use.

2.7 Comments and Recommendations

2.7.1 Potential Problems

During operations, a few issues were encountered. From time to time, a pipe, tube or connection would leak. In that instance, black plumbing tape (Magic Wrap) was used to secure and/or seal the tube until the next water change, when the tube could be replaced. This type of tape is advantageous because it can be used while the area is wet or leaking.

At one point, the tube between the large seawater reservoir pump and the supply plumbing came unhooked from the pump. The water sprayed directly up and out of the seawater reservoir. The seawater reservoir was found with approximately 60 liters water remaining. The pump was reattached using a hose clamp to ensure a secure connection, and the connection has not been problematic since. Such an event also highlights why having a nearby floor drain has been advantageous for running this system.

Algae growth was vigorous in some colony reservoirs, and several methods were employed to combat this nuisance. The sides of each colony reservoir were scraped with a piece

of the medium filter media prior to water changes to dislodge a majority of the algae. In addition, light levels were reduced to 25-50% using the dimmer switch. Any number of macro-algae can also be placed in the central part of the filtration subsystem with a light to remove nitrates/nitrites from the water, should it be an issue (see section on filtration subsystem construction for further discussion).

2.7.2 Avenues for Future Research

The intended use for this colony system was to supply subjects for experiments outside of the colony system itself (e.g. in petri dishes). Foraminiferal tests record various environmental parameters such as seawater temperature, salinity, $\delta^{18}O$, and Mg/Ca (Lear et al., 2000). In foraminifera, metabolism is based on taxonomic order (Pawlowski et al., 2013). While some studies have investigated the effects of changing seawater Mg/Ca and temperature on benthic foraminiferal test Mg/Ca (Bentov and Erez, 2006; Raitzsch et al., 2010; Mewes et al., 2014; Evans et al., 2015) only one of these (Evans et al., 2015) varied both Mg/Ca and temperatures in their experiments. Culture studies could be done to both better determine the effects of temperature, and also to better determine the effects of seawater Mg/Ca.

Additionally, several avenues of foraminiferal research can be taken. With our system, research can be done on foraminiferal growth, reproduction, and behavior. One study by Gooday investigated benthic foraminiferal response to phytodetritus and its role in foraminiferal reproduction (Gooday, 1988). This can be further investigated in many orders of foraminifera to see if all orders of foraminifera respond to phytodetritus by increasing reproduction rates.

Foraminiferal ecology can also be investigated, such as the effect of parasites on foraminiferal reproduction and growth rates. For example, in some of the specimens obtained from Long Island Sound, CT, USA, parasites were observed. Foraminifera possessing these parasites were removed from the system. However, little is currently known regarding how such parasites affect the livelihood of foraminifera. These parasites could purposely be introduced into foraminifera-containing petri dishes to see what the possible metabolic and morbidity effects of this parasite are.

Foraminifera also can be used as micro-recorders of environment pollution. A study by Morvan et al. (2004) used foraminifera to assess environmental remediation of oil spills in the Bay of Bourgneuf, France. Several studies can be conducted with this in mind, such as testing if oil slows foraminiferal growth rates, which species are more resistant to pollution, if any particular size is more resistant to the effects of an oil spill, industrial pollution, heavy metal contamination, etc. It is especially difficult to experiment with vertebrates due to lengthy Institutional Review processes. Using foraminifera, which are invertebrates, obviates the need for these processes.

Foraminifera are one of the world's major carbonate producers (Hallock, 1981). Carbonates act to buffer natural water systems, one of those systems being the ocean (Pytkowicz, 1967). Further research on foraminifera of all species will help us better understand their

physiology, ecological impact, and biochemical impacts on the world's oceans. Additionally, oceans are acidifying (Caldeira and Wickett, 2003). The effect of ocean acidification on foraminifera needs to be better understood due to the large role that foraminifera play in the world's ocean systems and carbon cycle.

2.7.3 Total Cost of Ownership

It cost approximately US$2400 to build the aforementioned system and it has cost approximately $840 per year to maintain. Water changes are most necessary when any test strip parameter is in the undesirable range, or when the system is low on water. Cost can be reduced somewhat by decreasing the frequency of water changes, but closer monitoring must be done to assure good foraminiferal health. Also, as long as the mechanical filter media is not stained or filled with organic detritus, it can be reused many times, further reducing cost of maintenance. If foraminifera are introduced with large amounts of cyanobacteria, this will clog filtration and make it difficult to reuse filters. Adding other biota to control algae is not advised, as they have been known feed on foraminifera (e.g. blenny fish).

If one wishes to have the smallest system, the cost of ownership is substantially lower with a total of US$82 to build the system and approximately $200 to maintain the system for one year.

2.7.4 Broader Impact

This system is easy to build and maintain, therefore, it can be deployed outside the research lab. This system could be used in K12 science classrooms to help students better visualize the workings of cells because Foraminifera possess some of the largest single cells. Species such as *Reticulomyxa*, a "naked" foraminifer that grows no test, would make it easier to see the internal machinery of cells. It also shows that some single cells can be relatively large, as opposed to human blood and organ cells, which is what most students see. The system could also be used to involve students in the scientific process by allowing them to design and conduct small experiments on how environmental changes affect organisms. Areas of investigation include things such as how salinity affects foraminiferal morbidity or how pollution affects test growth. In order to observe these organisms, students will also develop their microscopy skills. This expands the students' skills into nanoscience areas, which is currently a rapidly expanding area of science. This system can also help students learn about sedimentology and paleontology by being able to see both a pre- and post-fossilized version of an organism. At a very basic level, students that live far from the ocean could be exposed to the complexity and beauty of the microscopic inhabitants of seawater.

Outside of the realm of academia, there are several aquaculture hobbyists who could easily utilize this system. Several fish breeders could use systems like this to easily and quickly isolate pregnant fish so that they do not consume their own young. This system could be used for breeding smaller invertebrates as well, such as small shrimps, which are not suited for typical

aquariums with higher flow rates. Also, plant breeding can be conducted in this system, as several plants require different substrate, so breeding them in a single large aquarium proves difficult. Another frequent issue among aquaculture hobbyists is the desire to keep several fish that don't live peacefully in a communal setting. For example, this system would now make it possible for hobbyists to keep both shrimp and fish that eat those shrimp (such as a small loach or tetra) in the same system without the latter consuming the former.

FIGURES

Figure 1: Photograph of the foraminiferal colony system before foraminifera were added. Compare with Figure 2 taken after system had been in operation for one year.

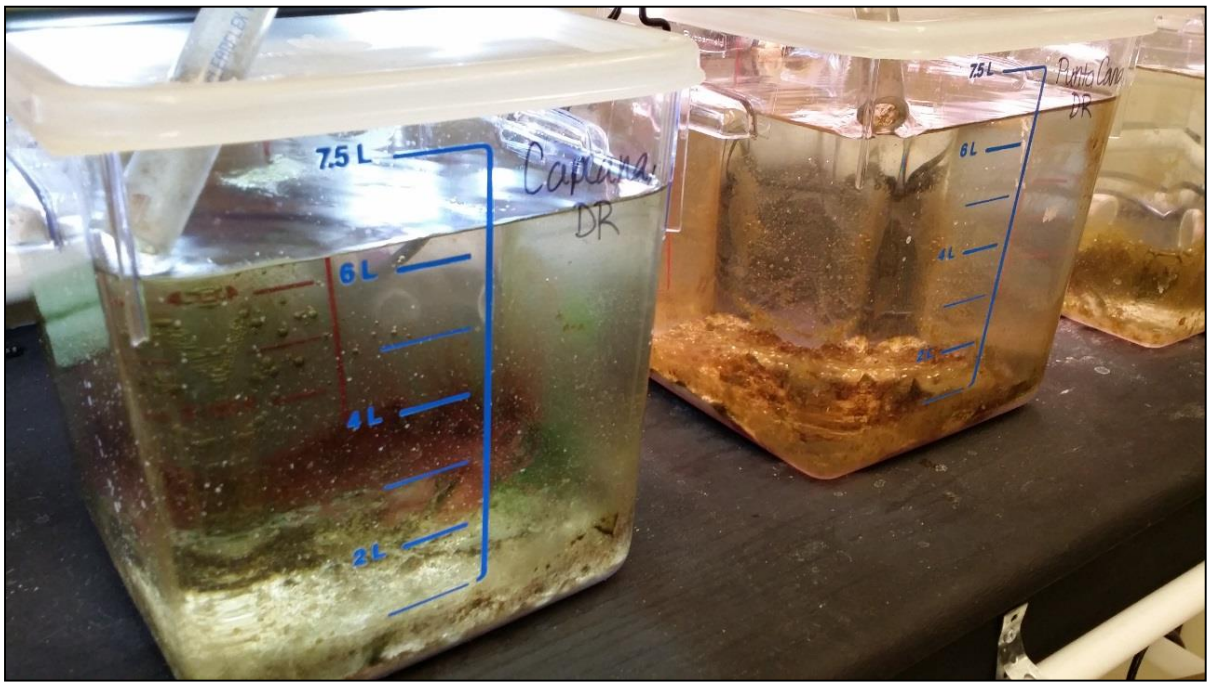

Figure 2: Photograph of colony reservoirs approximately one year after foraminifera were introduced, notice that different algal ecology is maintained in each colony reservoir.

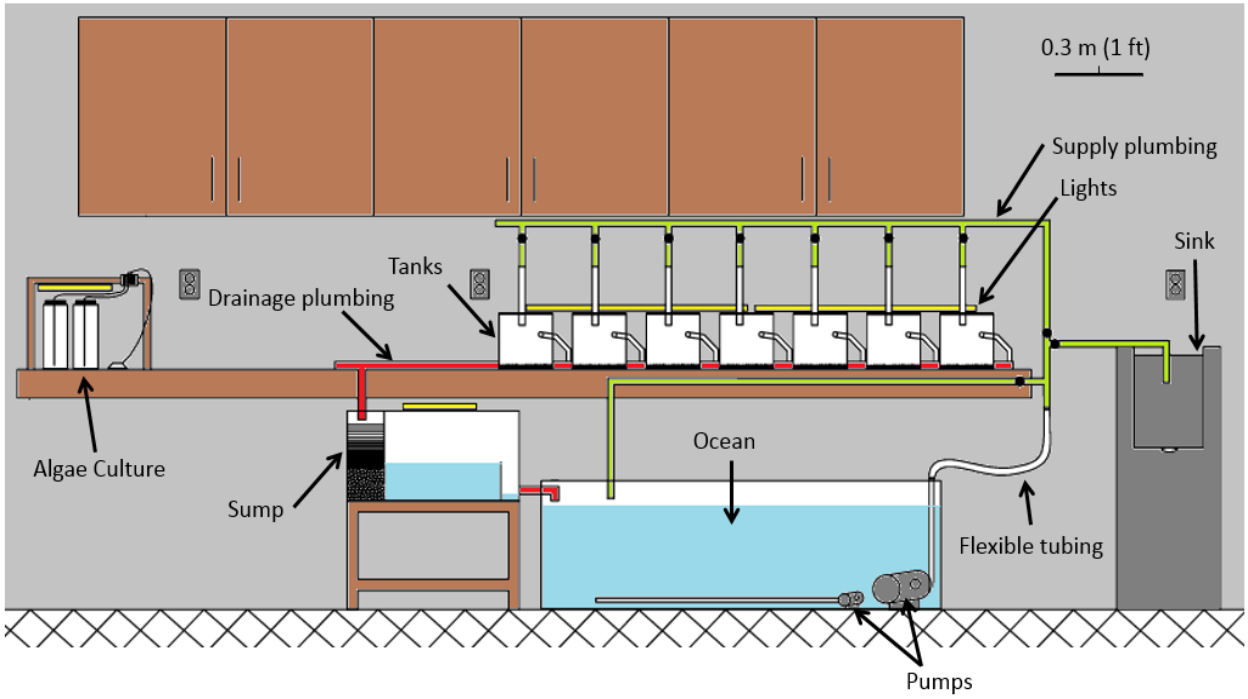

Figure 3: Scale drawing of foraminifera colony system. Lights are shown in yellow, drainage plumbing is shown in red, supply tubing is shown in green, and flexible tubing portions are shown in white.

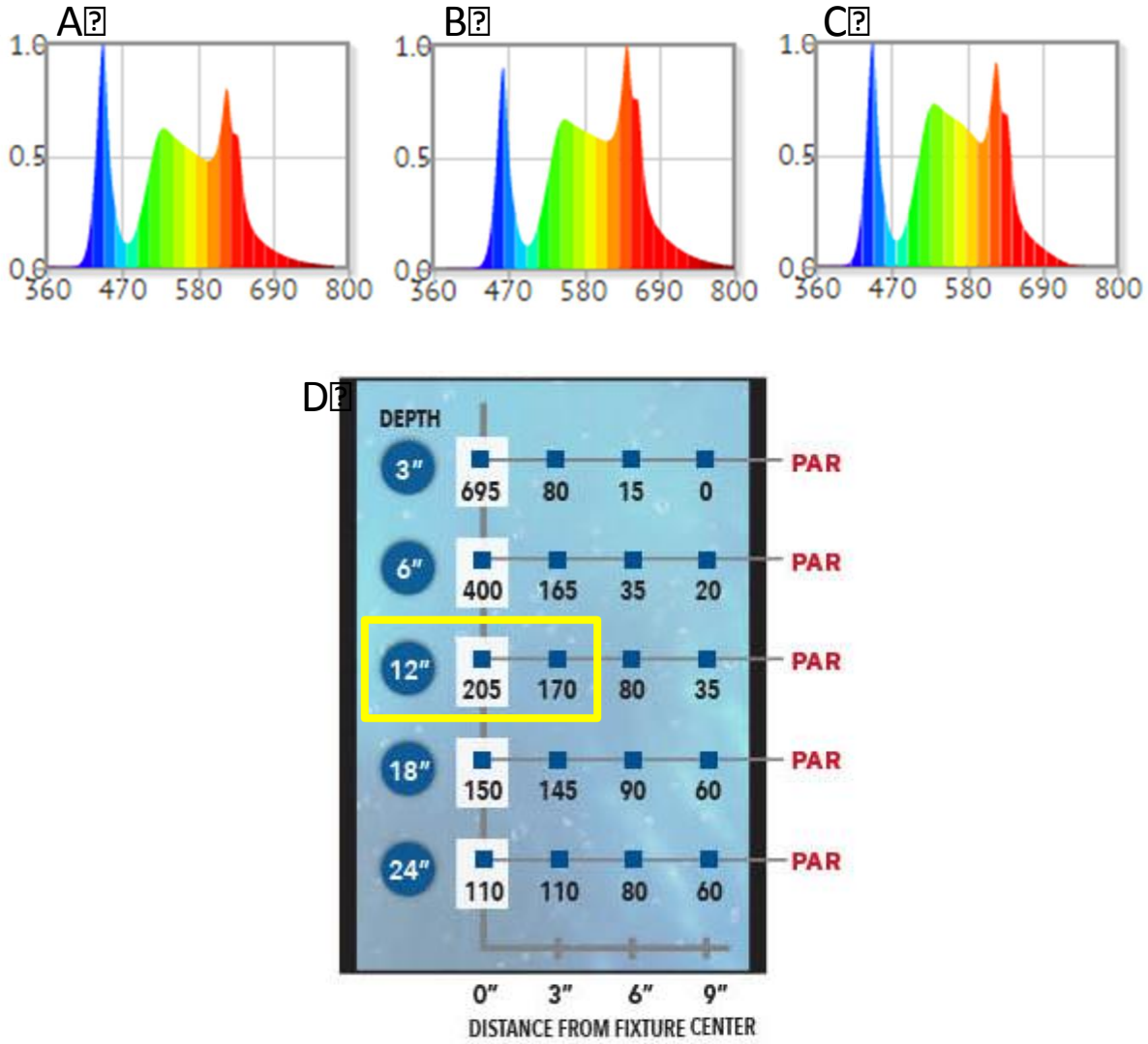

Figure 4: Attenuation of certain wavelengths in air and water. Electromagnetic spectrum (A) and photosynthetically active radiation (PAR), the radiation that photosynthesizing organisms can use. PAR is lost with water depth (C). D) PAR (in [µmol of photons]/m^2/s) at corresponding depths and distance laterally from a culture light with a 60° beam angle. The 12-inch depth is (outlined with a yellow box) because the grow light was situated 12 inches above the sediment substrate on which the foraminifera grew. Colony reservoirs had an 8-inch by 8-inch base, so foraminifera could be a maximum of 4 inches from fixture center. Image is modified from buildmyled.com.

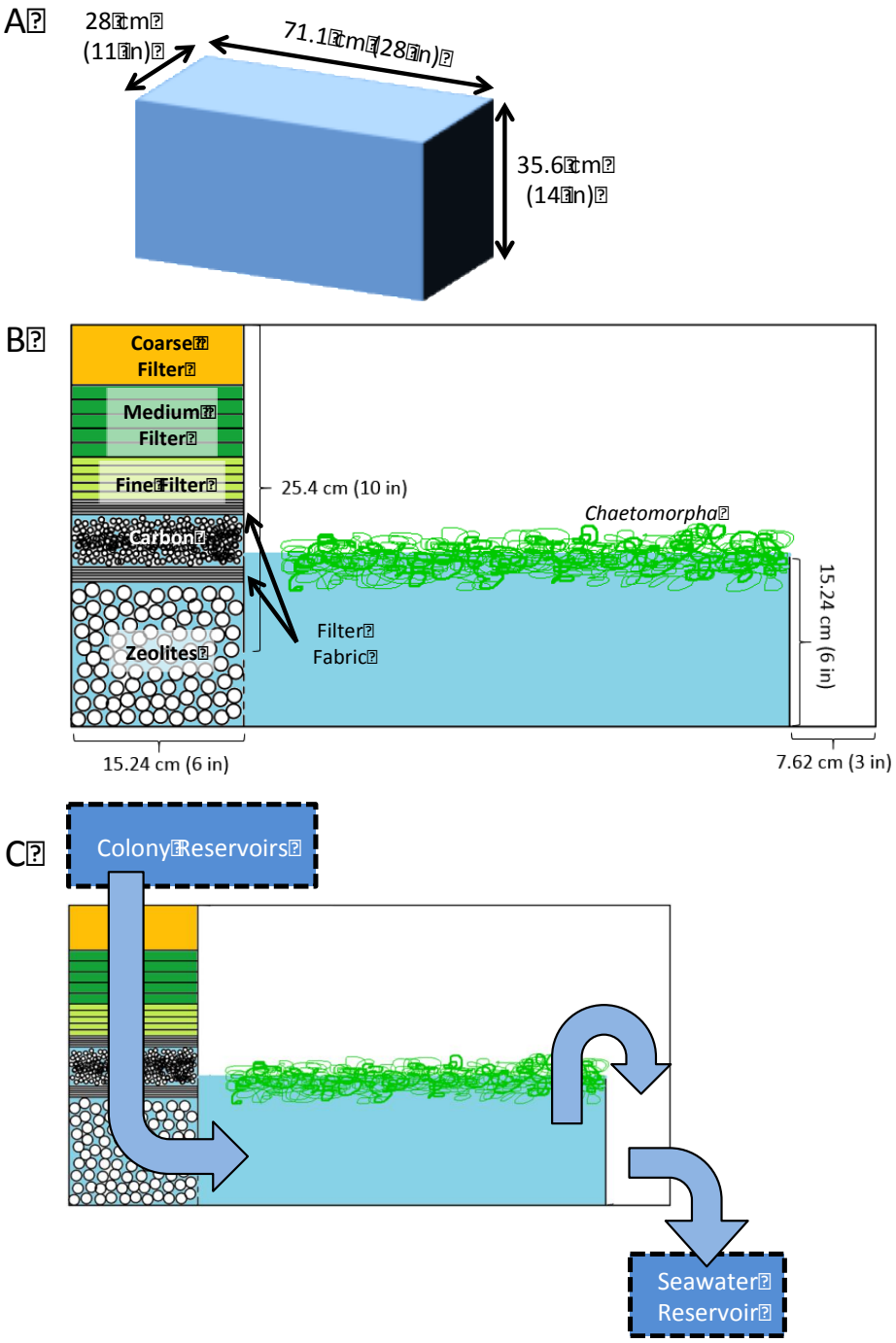

Figure 5: Scale diagram of filtration system. **A)** Three-dimensional perspective of filtration subsystem tank. **B)** Scale drawing on internal layout of filtration subsystem and description of physical/chemical filtration layers. **B)** Water flow shown with blue arrows.

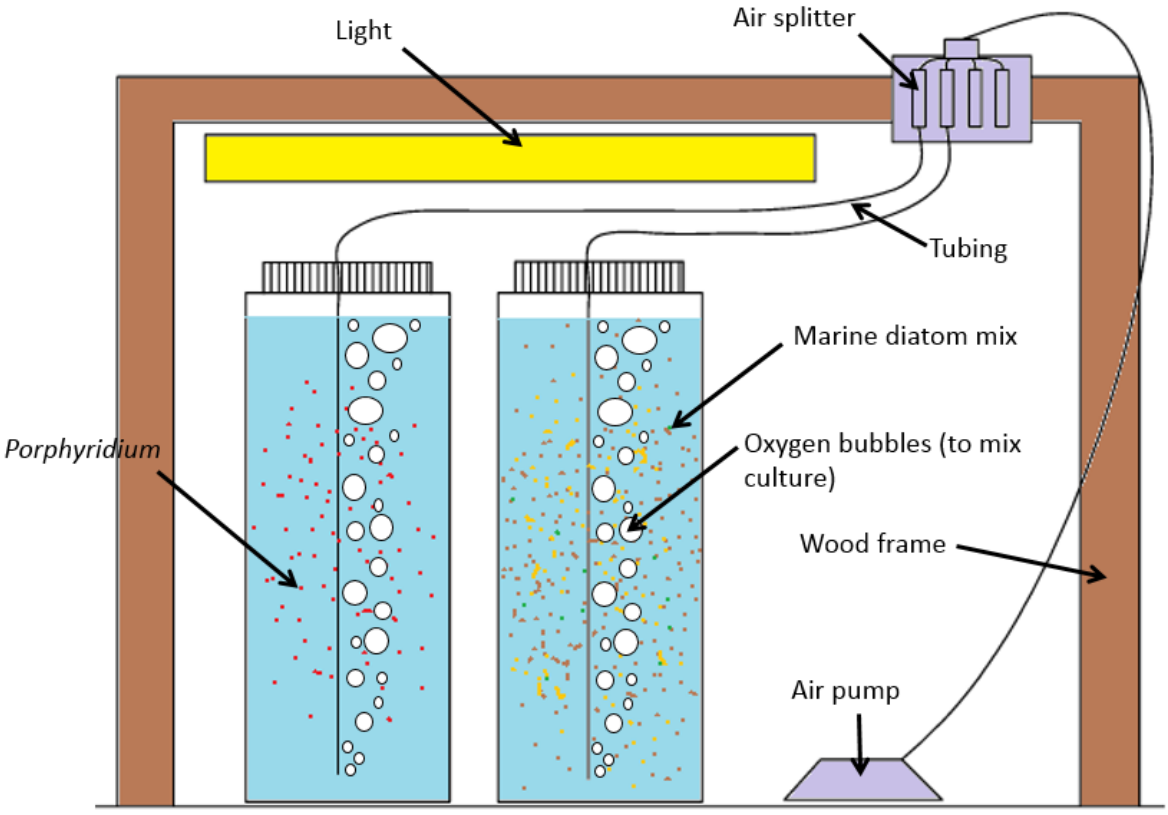

Figure 6: Diagram of algae culture. Air pump supplies oxygen gas to algae culture through tubing, light (shown in yellow) supplies spectrum ideal for *Porphyridium* (red algae).

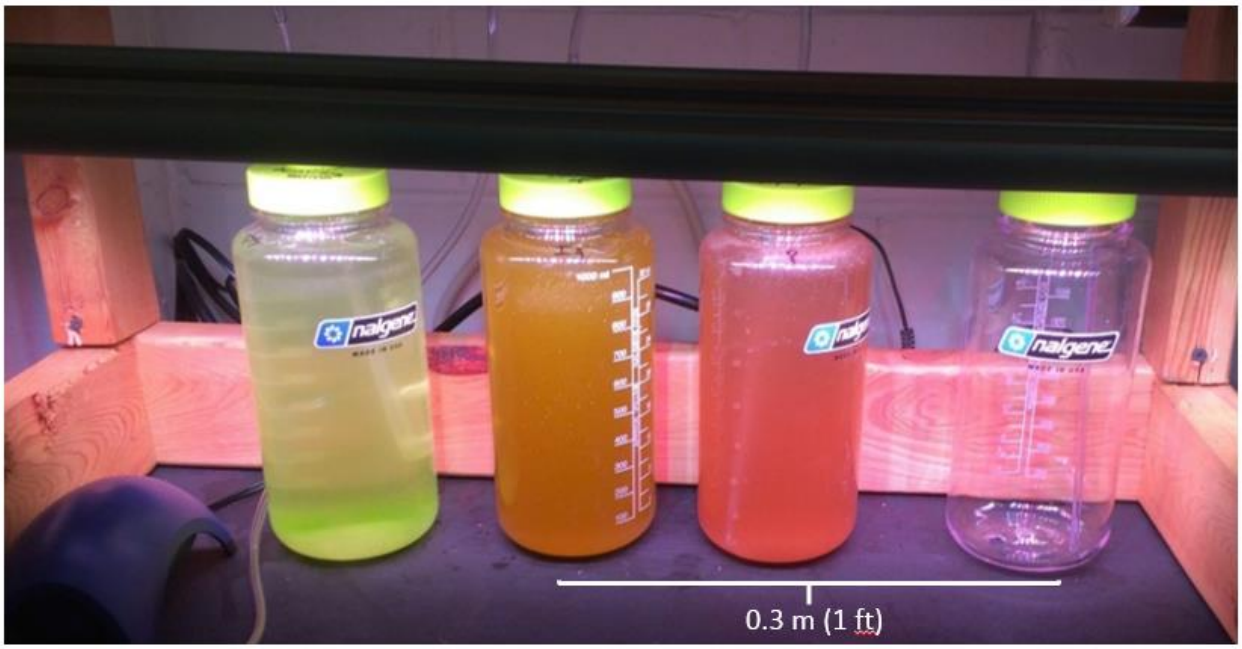

Figure 7: Photograph of algae culture in operation shows the *Porphyridium* (red) and the marine diatom mixture (brown and green) bottles. The air splitter is above the light (not shown in the photo). The air pump is situated in the lower left corner of this photograph.

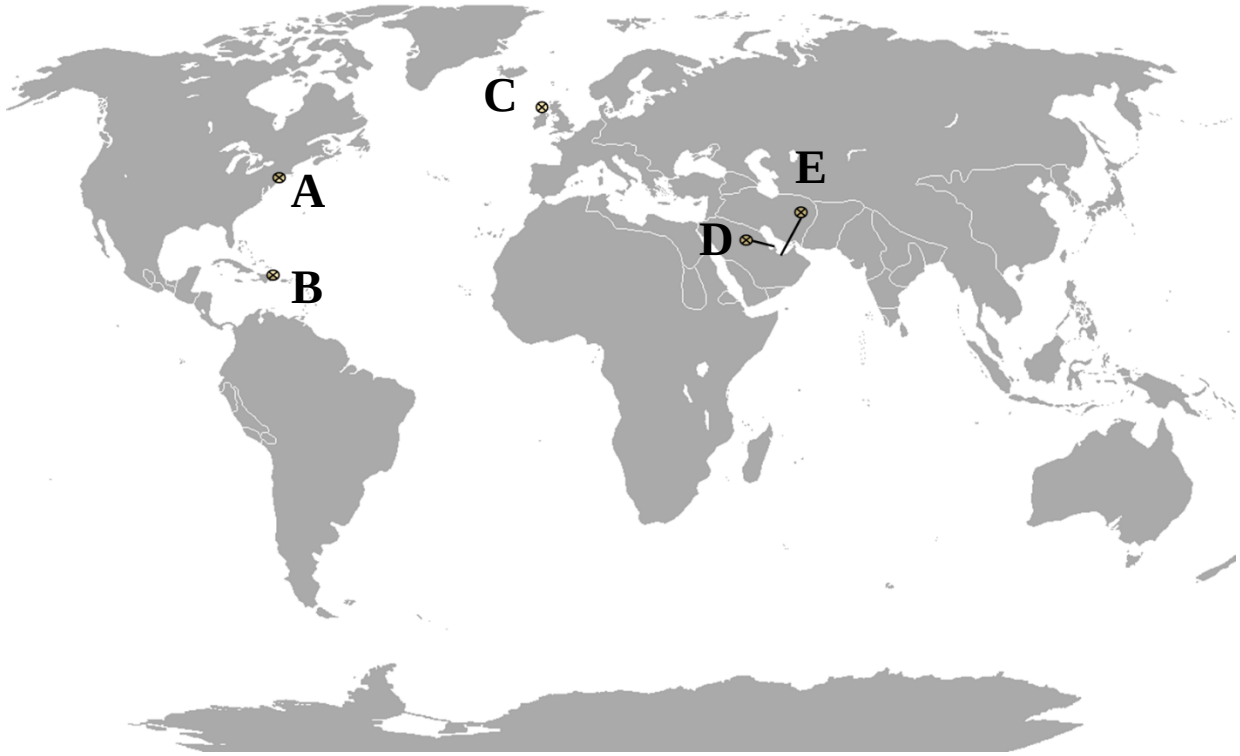

Figure 8: Map of foraminifera collection locations. The dots show where foraminifera were collected from A) Long Island Sound, off of Connecticut, USA; B) the Dominican Republic; C) off of the coast of Scotland; D) Al Thakira, Qatar; and E) Abu Dhabi, United Arab Emirates.

TABLES

Table 1. Foraminifera Culture System Supply List. All items, companies, and prices used to construct various components of the foraminifera culture system, algae culture, miscellaneous supplies, and smallest possible system.

Item	Manufacturer	Vendor	Item Number	Component	Cost Each (US$)	Quantity	Total (US$)
Tank Kit, 5 gallon	Marina	Amazon.com	15250A1	smallest system	54.99	1	54.99
Aquarium Heater	Tetra	Amazon.com	26447	smallest system	9.99	1	9.99
Instant Ocean Sea Salt (10 gallon)	Instant Ocean	Amazon.com	51378012003	smallest system	16.53	1	16.53
						COMPONENT SUBTOTAL	81.51
Tank	Pentair Aquatic Ecosystems	pentairaes.com	S103150	Seawater Reservoir	537.81	1	537.81
Lexane Lid	Iowa State University	n/a	n/a	Seawater Reservoir	30	1	30
Instant Ocean Sea Salt (200 gallon)	Instant Ocean	Amazon.com	SS1-200	Seawater Reservoir	40.49	1	40.49
						COMPONENT SUBTOTAL	608.3
Tank	Fisher Scientific	fishersci.com	03-484-23	Colony Reservoir	33.54	7	234.78
¾" Nylon Bulk Head Union	Generic	Local Hardware Store	n/a	Colony Reservoir	12.55	7	87.85
1 Foot of ¾" Vinyl Tubing Clear (Water Out)	Generic	Local Hardware Store	n/a	Colony Reservoir	1.61	7	11.27
Refugium-Red Algae Light, 24-inch	Build My LED	buildmyled.com	n/a	Colony Reservoir	179	3	537
Light Timer	Intermatic	Amazon.com	.'N311	Colony Reservoir	10	3	30
Manual Dimming Switch	Build My LED	buildmyled.com	n/a	Colony Reservoir	14.99	3	44.97

Table 1. Continued

1 Foot of ½" ID Vinyl Tubing (Water In)	Generic	Local Hardware Store	n/a	Colony Reservoir	1.47	12	17.64
						COMPONENT SUBTOTAL	963.51
Manual Dimming Switch	Build My LED	buildmyled.com	n/a	Filtration System	14.99	1	14.99
12 Inch Refugium-Red Algae Light	Build My LED	buildmyled.com	n/a	Filtration System	119	1	119
Filter Pad Fine Beige 24x24x2	Drs. Foster and Smith	drsfostersmith.com	CD-79424	Filtration System	12.59	1	12.59
Filter Pad Superfine Lime 24x24x1	Drs. Foster and Smith	drsfostersmith.com	CD-79422	Filtration System	9.09	1	9.09
Filter Pad Felt 30x36x1/8 100 µm	Drs. Foster and Smith	drsfostersmith.com	CD-79434	Filtration System	6.29	1	6.29
Filter Pad Coarse 24x24x1 Black	Drs. Foster and Smith	drsfostersmith.com	CD-79429	Filtration System	16.99	1	16.99
Fluval Biomax Filter Media 17.63oz	Drs. Foster and Smith	drsfostersmith.com	CD-27246	Filtration System	11.99	1	11.99
Activated Carbon Pelleted F&S Brand 4.73 L	Drs. Foster and Smith	drsfostersmith.com	CD-79118	Filtration System	78.69	1	78.69
Aquaclear Media Bag 500	Drs. Foster and Smith	drsfostersmith.com	CD-120529	Filtration System	3.99	2	7.98
Aquarium Safe Silicone Caulk	Aqueon	Amazon.com	15905650106	Filtration System	12.3	2	24.6
Chaetomorpha Algae (Medium)	Reefs 2 Go	reefs2go.com	n/a	Filtration System	29.99	2	59.98
Tank, 20 gallon	Pentair Aquatic Ecosystems	pentairaes.com	S103020	Filtration System	149.7	1	149.7
Lexane Lid	Iowa State University	n/a	n/a	Filtration System	0	1	0
						COMPONENT SUBTOTAL	511.89
Hose Clamps	Generic	Local Hardware Store	n/a	Plumbing	1.57	2	3.14
Plumbing Straps, ¾"	Generic	Local Hardware Store	n/a	Plumbing	7.09	1	7.09

Table 1. Continued

Screws 1/2"	Generic	Local Hardware Store	n/a	Plumbing	0.06	35	2.1
2000 gph Submersible Waterfall Pump	Smartpond	Local Hardware Store	WPR2000	Plumbing	159	1	159
330 gph Submersible Pond Pump	Smartpond	Local Hardware Store	DP330	Plumbing	57	1	57
1 Foot Of 3/4" Vinyl Tubing Clear For Pump	Generic	Local Hardware Store	n/a	Plumbing	1.61	3	4.83
½" Stop Waste Valve	Generic	Local Hardware Store	n/a	Plumbing	2.63	10	26.3
½" Cvpc 5' Pipe (Supply Plumbing)	Generic	Local Hardware Store	n/a	Plumbing	1.99	4	7.96
1¼ to ¾" Tee (Tank Drain T)	Generic	Local Hardware Store	n/a	Plumbing	2.8	3	8.4
1¼" Pipe 5' Section (Drain Plumbing)	Generic	Local Hardware Store	n/a	Plumbing	1.87	7	13.09
OR 3/8-1/2" Nylon Hose Barb MIP	Generic	Local Hardware Store	n/a	Plumbing	2.38	7	16.66
½" Tee	Generic	Local Hardware Store	n/a	Plumbing	0.24	8	1.92
90 Degree Street Elbow 1/2"	Generic	Local Hardware Store	n/a	Plumbing	0.41	3	1.23
1 1/4" Cap	Generic	Local Hardware Store	n/a	Plumbing	0.86	1	0.86
Special Female Adapter ½"	Generic	Local Hardware Store	n/a	Plumbing	0.71	1	0.71
1¼" to 1¼" Tee (Filtration Drain Tee)	Generic	Local Hardware Store	n/a	Plumbing	2.8	1	2.8
1¼" Elbow	Generic	Local Hardware Store	n/a	Plumbing	1.36	1	1.36
						COMPONENT SUBTOTAL	314.45
Refugium-Green Algae Light, 24-inch	Build My LED	buildmyled.com	n/a	Algae Culture	119	1	119
Manual Dimming Switch	Build My LED	buildmyled.com	n/a	Algae Culture	14.99	1	14.99

Table 1. Continued

Nalgene Bottles 32 Oz Wide Mouth Clear	Nalgene	Amazon.com	1PINTWM	Algae Culture	5.24	2	10.48
Aquarium Tubing (25 Ft)	Python	Amazon.com	25PAL	Algae Culture	5.9	1	5.9
Air Hose Splitter (2 Way, In Line)	IDS	Amazon.com	#13876-2#{3}	Algae Culture	3.99	1	3.99
10 Gallon Air Pum	Tetra	Amazon.com	77851	Algae Culture	8.49	1	8.49
Marine Diatom Mix	Carolina Biological	carolina.com	151367	Algae Culture	15.75	1	15.75
Porphyridium Mix	Carolina Biological	carolina.com	153599	Algae Culture	7.5	1	7.5
F2 Culture Media, Micro Algae Grow	Florida Aqua Farms inc	florida-auqa-farms.com	FA-MIS	Algae Culture	6.5	1	6.5
Stiff Air Hose	Lee's pet products	Amazon.com	107127	Algae Culture	2.54	3	7.62
						COMPONENT SUBTOTAL	200.22
Magic Tape	Generic	Local Hardware Store	n/a	Tools and Maintenance	9.78	1	9.78
PVC Medium Solvent Cement	Generic	Local Hardware Store	n/a	Tools and Maintenance	4.44	1	4.44
PVC Pipe Cleaner	Generic	Local Hardware Store	n/a	Tools and Maintenance	5.48	1	5.48
Salinity Probe	Extech				649.00	1	649.00
						COMPONENT SUBTOTAL	668.70

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CHAPTER III

GROWTH OF FORAMINIFERA IN SEAWATER WITH VARYING WATER MG/CA AND TEMPERATURES TO AID IN CALIBRATION OF THE BENTHIC FORAMINIFERAL CENOZOIC PALEOCRYOMETER

A Manuscript to be submitted to Geochimica et Cosmochimica Acta

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3.1 Abstract

The Cenozoic as a whole has been a time of global cooling. The story of this cooling, especially the timing and extents of continental glaciation, remains clouded due to conflicting evidence of Oligocene ice sheets, such as deepening of the carbonate compensation depth, and presence of ice rafted debris at northern latitudes (Zachos et al., 2001; Tripati et al., 2008; Dawber et al., 2011). To better understand the complexity of this transition, better calibrations of new paleoclimate proxies are needed. The Mg/Ca of foraminiferal calcite has been shown to be an accurate proxy for paleo-seawater temperature (T), which can be used to decipher the timing of glaciations when combined with foraminiferal calcite $\delta^{18}O$ (Martin et al., 2002). However, over long time scales (greater than 10^6 years), these paleo-thermometers must account for changes in the seawater Mg/Ca caused by submarine alteration of basalt extruding at mid-ocean ridges. While numerous core-top and experimental studies have examined the effect of temperature on foraminiferal shell Mg/Ca, few experimentally calibrated models explain how Mg/Ca of benthic foraminifera respond to variations of seawater T and Mg/Ca. To this end, multiple specimens of a benthic foraminifer were grown in different environmental conditions (e.g. water T and Mg/Ca) in individual cultures. A power function was obtained that models the sensitivity of foraminiferal shell Mg/Ca to water Mg/Ca for the third chamber of growth of the benthic foraminifera *Peneroplis planatus* at 25°C. Applying these calibrations to recent studies (e.g. Segev and Erez, 2006; Raitzsch et al., 2010; Mewes et al., 2014; Evans et al., 2015) and past literature will help produce a portion of a more accurate model for paleo-thermometry over deep time (e.g. the past 50 My). This analysis confirms the recently reported results for the effect of seawater Mg/Ca on foraminifer Mg/Ca in high-Mg foraminifera at 25°C.

3.2 Introduction

Past attempts at deciphering the history of the transition from the Cretaceous-Early Cenozoic Greenhouse climate to the late Cenozoic ice house climate over the last 50 million years have disagreed over the timing and extents of continental glaciation, with some favoring Northern Hemisphere glaciation only in the Pleistocene (Lisiecki and Raymo, 2005; Zachos et al., 2001). Others have identified signs of Northern Hemisphere glaciation as far back as the

Eocene (e.g. Tripati et al., 2008) based on the presence of ice rafted debris in marine sediments. To help clear up these discrepancies, foraminiferal response to seawater Mg/Ca and temperature can be combined with other data (like long-term trends in foraminiferal $\delta^{18}O$ and seawater Mg/Ca) to generate a times series for continental glaciations, global sea level, and climate over deep time (e.g. Lear, 2000). Benthic foraminifera serve as an accurate indicator of bulk ocean properties, because they inhabit deep-ocean water masses less affected by high-frequency variations in sea surface temperature and salinity.

The response of foraminiferal calcite to both seawater Mg/Ca and temperature can be tested in the laboratory through a set of culture experiments that vary seawater Mg/Ca and temperature. Attempts have been made to generate deep-time records of Cenozoic seawater $\delta^{18}O$ using foraminiferal Mg/Ca as a proxy for seawater temperature (e.g. Lear et al., 2000, 2008). These studies routinely employ a Mg/Ca paleothermometer that does not accurately account for 1) secular variation in seawater Mg/Ca, and 2) how seawater Mg/Ca affects test Mg/Ca (Ries et al., 2004; Hasiuk and Lohmann, 2010). The paucity of culture studies that investigate the effect of changing seawater Mg/Ca on test Mg/Ca contributes to this continued lack of understanding.

Benthic foraminifera are useful in deep-time paleoclimate studies because they inhabit deep-ocean water masses that are less affected by high-frequency variations in ocean properties (like sea surface temperature and salinity). To test this relationship, the three main foraminiferal test chemistries must be investigated and a function determined. The earliest study which investigates the relationship between foraminiferal Mg/Ca and seawater Mg/Ca was conducted by Segev and Erez (2006). Intermediate-Mg calcite (IMC), benthic *Amphistegina lessonii* and *Amphistegina lobifera* were used for this study. Foraminifera were cultured for 52 days in Erlenmeyer flasks kept in a 24°C water bath. The culture medium was a mix of artificial and natural seawater, with Mg/Ca ratios of 0.5, 1.0, 2.5, 5.0, 7.5, and 10 (Segev and Erez, 2006), spanning the suggested Phanerozoic variation in this property (Hardie, 1996). Water was changed two times a week and specimens were kept in the dark. Foraminifera were not fed during the study (Segev and Erez, 2006). Geochemical analysis was done via electron microprobe (EMP) and a correlation and transfer function were obtained (Figure 9). Both taxa were grown at only a single temperature.

More recently studies have been done on both low-Mg calcite (LMC) and high-Mg calcite (HMC) foraminifera. *Heterostegina depressa* and *Ammonia tepida* were cultured in covered containers for 2-4 weeks (Raitzsch et al., 2010). *A. tepida* was cultured in darkness 24 hours a day in the dark in an incubator at 18°C; it did not need light exposure due to absence of endosymbionts. *H. depressa* was kept in an incubator at 24°C with a 12-hour light/12-hour dark cycle. Seawater was a mix of natural seawater and artificial seawater with Mg/Ca ratios of 5.17, 5.56, 5.96, and 6.20. Geochemical analysis was done using laser ablation inductively couple mass spectrometry and a transfer function was obtained for each taxon that explained how foraminifer Mg/Ca varied as a function of seawater Mg/Ca (Figure 9). The utility of these transfer functions is limited because the taxa were grown over such a small range in seawater

Mg/Ca (5.17 - 6.2). Like Segev and Erez (2006), both taxa were grown at only a single temperature.

A second study was done on the IMC benthic foraminifera *A. lessonii* and *Ammonia aomoriensis* (Mewes et al., 2014). Foraminifera were cultured in petri dishes in seawater with Mg/Ca of 1.0, 5.0, and 10.0 at 25°C. Foraminifera remained in culture until reproduction. When juveniles were produced, they were kept in culture until they had 3-5 new chambers at which point they were euthanized. Foraminifera were analyzed using laser ablation inductively coupled mass spectrometry and a transfer function was created from the data (Figure 9). Like the previous studies, both taxa were grown at only a single temperature.

Operculina ammonoides, a HMC benthic foraminifer, was cultured in Erlenmeyer flasks placed in water baths with artificial seawater Mg/Ca of 5.42, 6.82, 5.33, 4.28, 3.34, and 2.27 (Evans et al., 2015). Seawater was kept at 24°C. Seawater with Mg/Ca 5.42 was also cultured at 19, 21, 22.5, 24, 25.6, and 27°C. Water was changed every second day. Salinity varied from 37-40.7‰, alkalinity varied from 2.175-2.505 mg/L, and pH varied from 7.98-8.14. After approximately 65 days, foraminifera were euthanized and analyzed using laser ablation inductively coupled plasma mass spectrometry. A transform function was generated that took accounted for variation in water temperature and water Mg/Ca (Figure 9).

Like Evans et al. (2015), this study also uses a high-Mg calcite foraminifer, *Peneroplis planatus*. This is a large (some up to 4 mm in size), HMC benthic, milliolid foraminifer that hosts rhodophycean endosymbionts (Lee, 1990). The Mg/Ca range of *P. planatus* is 90 to 200 mmol/mol (Ponder and Glendinning, 1974). It was readily available in large numbers and easily identifiable due to its large size in the collection area. It was discovered that peneroplids thrived in the culture system designed by Jennings and Hasiuk (cf. Chapter II). Three reasons might explain the suitability of the colony system for Peneroplid horticulture. First, Instant Ocean has slightly higher Mg/Ca ratio (5.44) than natural seawater (approximately 5.2). In addition, the emission spectrum of the light fixtures employed in the colony system were designed specifically for the endosymbionts commonly found in peneroplids. Peneroplids also seem to prefer the marine diatom mix and the *Porphyridium* algae that were initially inoculated into the system. Also, hair algae grew readily in our system, which the peneroplids climbed on frequently.

3.3 Methods

3.3.1 Species Selection and Collection

Peneroplid planatus and adjacent sediment were collected during low tide from a shallow intertidal zone at Kite Beach, Abu Dhabi, UAE (latitude 24° 30' 48.93" N and longitude 54° 32' 54.42" E). *P. planatus* specimens were easily identified because of their large size (most around 1 mm in diameter) and color (specimens were white against the generally gray sediment color). Foraminifera were not sieved on site. They were transported in plastic jars to Ames, Iowa, USA. The specimens were transferred to the foraminiferal colony described in Chapter II approximately three days after collection. The Mg/Ca ratio of the seawater of the collection site

was approximately 5.2 and the salinity was 38 ppt; Mg/Ca ratio was measured from seawater by titration and salinity was measure with a digital salinity meter (Extech DO700).

3.3.2 Foraminifera Preparation

Once acclimated to the colony system, assumed to be after approximately two days (as the standard acclamation time for larger, more sensitive vertebrates is only half an hour), foraminifera-containing sediment was sieved over a 300-micron sieve. Sediment retained on the sieve was transferred to a petri dish and *P. planatus* were picked with a size 0 brush. Foraminifera were placed in a petri dish containing artificial seawater approximating natural seawater (mixed from Instant Ocean Seawater Mix) and 15 mg/L calcein, a calcite staining agent (Kurtarkar et al., 2015). After a dish had approximately 100 *P. planatus*, it was covered and placed in an incubator (ThermoFisher model 146E) set at 25°C for one week. This length of time would allow the foraminifera to uptake the calcein into its entire test (Kurtarkar et al., 2015). Water was changed after three days to keep assumed waste levels low, salinity constant, and replenish dissolved ions that had been used by the foraminifera. Ten dishes of foraminifera were picked in this manner for a total of approximately 1000 specimens.

3.3.3 Experimental Process

In order to vary water Mg/Ca, seawater mixtures of different chemistries had to be prepared from constituent chemicals rather than using a commercially available mix (like Instant Ocean). Seawater was mixed based on Formula 124 (Kester, Duedall, Connors, and Pytkowicz's recipe in Bidwell and Spotte, 1985). This recipe was used due to its successful track record in previously reported culture studies (e.g. Ries, 2004). Magnesium and calcium levels were altered to achieve desired Mg/Ca ratio. Appendix A provides details of the artificial seawater recipes used in this study.

Seawater was mixed in batches of 3 L per Mg/Ca chemistry in a multistep process for all experiments done. Most of the gravimetric salts (salts that are not hydrated: NaCl, KCl, Na_2SO_4, NaF) were dried at 150°C for approximately two weeks prior to mixing. Gravimetric salts, except NaCl, were then weighed and added to 2 L of water. Hydrated salts ($SrCl_2 \cdot 6H_2O$, $CaCl_2 \cdot 6H_2O$, and $MgCl_2 \cdot 6H_2O$) were weighed and added to the remaining water in a separate container. Hydrated salt solution was mixed into the gravimetric salt solution. Salinity was measured and NaCl was added until salinity of 30 ppt was reached (when salinity was raised higher than 30 ppt, it was difficult to keep all salts into solution). The exact amount of sodium chloride added varied with each mixture, but was approximately 72 g (cf. Appendix A).

Foraminifera that survived calcein staining were picked from isolation dishes and introduced to 0.5 L jars containing the various Mg/Ca ratios (0.6, 0.8, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0). After a jar had twenty foraminifera in it, it was placed lid side down in an incubator (ThermoFisher model 146E) that was set to the desired temperature (3°C, 12.5°C, and 25°C). See Table 2 for a summary of Mg/Ca and T selections for this experiment.

Each incubator contained an algae growth light specific to the spectrum used by red endosymbiont algae. Lights were set to a 12-hour on/12-hour off cycle. Each incubator contained ten jars, all placed equidistant from the light source. A Tidbit (Onset Hobo Dataloggers, Bourne, MA, USA) temperature monitor was placed in the center of the refrigerator to monitor temperature. Foraminifera were not fed during this experiment because diatom chloroplasts can be retained for weeks (Correia and Lee, 2000). Foraminifera were full and brightly colored, and thus assumed to be full of endosymbionts.

The foraminifera were kept in culture for 41 days. This duration allowed enough time for each foraminifer to grow at least one new chamber. At the end of the 41-day period, foraminifera were picked out of experimental seawater, dipped in ethanol, rinsed with deionized water, and left to desiccate. They were then observed under fluorescent light with an Olympus DP73 microscope to assess the number of newly grown chambers. Growth was assessed by the presence and number of non-fluorescent chambers that grew beyond fluorescent chambers. Foraminifera that did not fluoresce on any portion of their test were excluded from geochemical analysis because this suggests that they were likely not alive at the beginning of the experiment, as they did not uptake any calcein into their test (Berhnard et al., 2004). All live foraminifera either take calcein into the entire present test, or incorporate calcein into newly formed chambers (Bernhard et al., 2004). In either case, the new growth is easily distinguished from the old growth.

3.4 Geochemical Analysis of Experimentally Grown Foraminiferal Calcite

Foraminifera were chosen for analysis based on morphological size, number of new chambers, and presence of fluorescent chambers. All foraminifera chosen for analysis were approximately the same size (1 mm in diameter), in order to rule out Mg/Ca variations as a result of size differences (Elderfield et al., 2002). All foraminifera chosen for geochemical analysis grew at least three new chambers (Table 4), and a minimum of fifteen points of geochemistry were collected from these foraminifera (three per chamber on each of the three experimentally grown chambers, and three per chamber on two pre-experimental chambers). Multiple analyses per chamber allowed intra-chamber variations in Mg/Ca to be assessed.

Foraminifera were analyzed by electron microprobe analysis (EMPA) on a JEOL JXA-8230 Electron Probe Microanalyzer on 25-26 May 2015 (Department of Earth and Environmental Science, University of Iowa, Iowa City, Iowa, USA). Twenty foraminifera were placed in epoxy on each of five one-inch plugs and polished. Magnesium, calcium, iron, strontium, manganese, and barium were measured as weight percent oxides and elemental ratios were computed from that data. In house, high purity standards were used to calibrate the EMP and consisted of dolomite for Mg, calcite for Ca, pure iron metal for Fe, celestite for Sr, and bustamite for Mn.

Qualitative X-ray mapping of elemental chemistry was done on a total of 102 foraminifera (approximately three from each jar). This mapping allowed us to select areas of

homogeneous chemical composition for quantitative point analyses. Quantitative point analyses were done on 18 foraminifera. Foraminifera that grew the most new chambers and were the least damaged by epoxy polishing were chosen for quantitative point analysis. Points were chosen in areas that showed the most homogeneous composition on qualitative x-ray maps. A 5-micron electron beam was used; the depth at which the beam penetrated varied based on composition, with softer minerals being removed more easily than harder minerals. The electron beam did not penetrate deeper than approximately 10 microns at any time. Quantitative point geochemical analyses were made on all new chambers and at least two pre-experiment chambers to document chamber chemistry change from pre-experiment to experimental conditions. Only foraminifera that grew three or more new chambers were selected for point analysis. This is more than previous studies, which show fewer new chamber analyses (Raitzsch et al., 2010; Evans et al., 2015).

3.4.1 Errors

Errors were calculated in all areas possible. Error bars are too small to show on any figures. Water Mg/Ca error could result from contamination from trace amounts of Mg or Ca in stock chemicals (usually <0.01 wt%). These were converted to the maximum and minimum atomic ratios that resulted in maximum error of ±0.05 mol/mol for the seawater Mg/Ca. For the EMP analysis, errors were also calculated and were given in percent of wt% oxides (0.1% on MgO and 0.3% on CaO). This was converted again to the maximum and minimum atomic ratios that resulted in the maximum error of ±1.1 mmol/mol foraminiferal Mg/Ca.

Temperature in incubators varied from 3.04-3.99°C for the 3°C incubator, between 13.43-16.13°C for the 12.5°C incubator, and between 23.01-25.21°C for the 25°C incubator. The temperature in the 3°C incubator reached a temperature of 6.23 during one water change. The error for these monitors is ±0.2°C.

3.5 Results

3.5.1 Growth

In order to evaluate the effect of varying seawater temperature and Mg/Ca on test Mg/Ca using adult foraminifera, it was important to be able to identify and analyze new chambers grown under experimental conditions. Foraminifera do not modify the Mg/Ca of chambers that have already calcified (Hemleben et al., 1986).

At least one geochemical analysis was obtained from each chamber. However, most new growth chambers were large enough and survived sample preparation with sufficient integrity to that three points were able to be analyzed on different parts of the new growth (inside, middle, and outside with respect to spiral growth axis) of the chamber (Appendix C). This allows an average composition for each chamber to be calculated as well as allowing for identification and rejection of outlier data.

All but three foraminifera appeared to survive the length of the experimental period (41 days). These foraminifera were still included in the experiment because cytoplasm was still present in the tests, even though those tests did not fluoresce. Overall, 161 (27%) foraminifera grew at least one chamber under the experimental conditions and 439 foraminifera (73%) either did not grow during experimental conditions, or they did not survive the experimental period. 110 (18%) foraminifera grew at least two new chambers in experimental conditions, 51 (9%) foraminifera grew at least three new chambers, and 16 (3%) grew more than three new chambers. A total of 102 foraminifera were selected for geochemical analysis (Table 3).

3.5.2 Abiotic Carbonate Precipitation

Over the course of the experiment, jars with the lowest Mg/Ca (0.6 and 0.8) precipitated aragonite crystals (Figure 10), regardless of which temperature water they were placed in. This likely altered the seawater chemistry, thereby making correlation to foraminiferal Mg/Ca more difficult. In order to eliminate the precipitation, the pH of the seawater would have had to be lowered to less than 7 to dissolve the aragonite. The seawater at the end of the experiment likely had a much higher Mg/Ca ratio than at the beginning for jars with seawater Mg/Ca of 0.6 and 0.8.

3.5.3 Qualitative Chemistry Mapping

102 specimens were qualitatively mapped for Mg, Ca, Mn, Sr, and Fe. Figure 11 shows the variability of calcium across many of the foraminiferal tests. Mg and Ca are not homogenously distributed throughout a single chamber or indeed the entire foraminifera. New growth has a different geochemistry than old growth. These maps helped decide which foraminifera would be point mapped for specific Mg, Ca, Mn, Sr, and Fe levels. Points were chosen to avoid areas with anomalously high or low Mg or Ca values.

3.5.4 Quantitative Chemistry at Points

Quantitative chemistry was measured at 276 points on 18 foraminifera (Appendix B, Appendix C shows these data mapped on SEM photomicrographs of individual foraminifera). Intra- and inter-chamber variation in Mg/Ca was observed (Appendices B and C); however, overall trends suggest that overall test chemistry was responding to changes in water chemistry.

The quantitative data show that at 25°C pre-experimental “old growth” Mg/Ca has no correlation with water Mg/Ca, whereas experimental Mg/Ca measured at the third chamber of “new growth” shows a very strong correlation water Mg/Ca (Figures 12 and 13). Higher Mg/Ca values in the seawater corresponded to higher Mg/Ca in the foraminifera. Conversely, lower Mg/Ca values corresponded to lower Mg/Ca values in the foraminifera (Figure 14). No error bars are shown for these data because they are eclipsed by the spot size in each figure.

Specimens grown at 12.5°C show opposite behavior—new growth in higher Mg/Ca have lower test Mg/Ca (Figure 15). Foraminifera verifiably grew in experimental conditions, due to

the presence of new chambers when viewed under fluorescence. However, seawater Mg/Ca of 0.6 and 0.8 precipitated aragonite crystals, as previously stated.

Specimens grown at 3°C show mixed behavior (Figure 16). Test Mg/Ca decreased when water Mg/Ca was below natural seawater (Mg/Ca = 5.2 mol/mol) and increased when water Mg/Ca was greater than natural seawater. However, test Mg/Ca was higher for water Mg/Ca of 6 than when water Mg/Ca was 8. Additionally, test Mg/Ca was lower for water Mg/Ca of 1 than when water Mg/Ca was 0.6.

The original objective of this experimental procedure was to determine how temperature and seawater Mg/Ca affect foraminiferal Mg/Ca. Linear regressions were done with available data. This regression was derived using data from the third chamber grown under experimental conditions. Using a linear model assuming an intercept of zero, data from the 25°C cultures show a very strong and significant trend ($r^2 = 0.99$, m = 0.023, m_SE = 0.001, $m_p = 2.8 \times 10^{-19}$, Figure 17, Equation 1).

$$Mg/Ca_{(foram)} = 0.023\ (Mg/Ca_{sw}) \qquad (1)$$

Using a power law model, which by definition has a zero-intercept and has been shown to be more broadly applicable to calcifying marine invertebrates (Hasiuk and Lohmann, 2010), data from the 25°C cultures still show a very strong and significant trend ($r^2 = 0.95$, F = 0.051, F_SE = 0.024, $F_p = 1.5 \times 10^{-17}$, H = 0.56, H_SE = 0.03, $H_p = 9.2 \times 10^{-11}$, Figure 17, Equation 2).

$$Mg/Ca_{(foram)} = 0.05\ (Mg/Ca_{sw})^{0.56} \qquad (2)$$

When looking at the 0^{th} chamber (the last chamber grown before experimental conditions), there is no trend in foraminiferal calcite Mg/Ca ($r^2 = 0.04$). This suggests an obvious and predictable foraminiferal response of test Mg/Ca to experimental conditions.

A second linear regression was done for the 3°C temperature set (Figure 13). At the beginning of this experiment, each foraminifer started with a slightly different test chemistry. The trend was a decreasing beginning foraminiferal Mg/Ca with increasing seawater Mg/Ca ($r^2 = 0.93$). At the new 3^{rd} chamber, the slope reverses in direction, showing a positive trend in the expected direction (increasing test Mg/Ca with increasing seawater Mg/Ca, $r^2=0.56$). This unexpected and complicated response is not as simple and robust as that provided by the 25°C data set. A power law function was not calculated for the 3°C temperature set.

A linear regression was not attempted for the 12.5°C temperature set due to a lack of specimens having a 3^{rd} chamber to analyze. Data from the 2^{nd} new chamber was not used because it is not likely that the foraminifera had purged all of their stored ions by that time (evidenced by the third chamber having a significantly different Mg/Ca ratio in all other foraminifera).

3.5.5 Other Experiments

Other experiments were attempted prior to the experiment documented in this chapter. These experiments used *Elphidium excavatum* and *P. planatus* (Appendices E and F) and artificially mixed seawater (Appendix D). These experiments did not yield any new, un-mutated growth, and therefore, were considered to be unsuccessful.

3.6 Discussion

3.6.1 Equilibration with Experimental Conditions

The third chamber grown under experimental conditions was used for this regression because it had the longest time to equilibrate with water chemistry. It is likely that ions stored in the foraminifer have also been used up by this point and all new chambers are grown using the experimental fluid. Other research (Hasiuk and Lohmann, 2010; Mewes et al., 2014) has shown that among calcifying organisms, this relationship is usually a power law. Power law regression results in a fit with an r^2 =0.91. Even though this is a lower r^2, it is a more reasonable fit because the equation is forced through the origin as might be reasonably assumed (an organism can't incorporate Mg in its test if no Mg is present).

This study was done on benthic foraminifera because are less affected by high frequency, cyclical variations in water masses due to their longevity—they live weeks to years while planktonic foraminifera live months (Sen Gupta, 1999). Though all foraminifera are similar in their metabolism, there are fundamental differences that account for the diversity of taxa and their suitability to different environments (Sen Gupta, 1999). This power law regression and information cannot be applied directly to planktonic foraminifera. Planktonic foraminifera have been shown to exhibit intra-chamber Mg banding (Spero et al., 2015). However, as milliolid benthic foraminifera likely metabolize similarly to each other, the power law regression derived above (Equation 2) can be applied for use with other milliolids.

3.6.2 Intra-test heterogeneity

As expected, it was observed that test Mg/Ca increased with increasing seawater Mg/Ca (Ries, 2004; Evans et al., 2015). Peneroplids grew faster than expected. Based on other studies, we expected approximately one chamber every two weeks with a total of three chambers of new growth (Kuile and Erez, 1984). However, some foraminifera grew as many as five chambers and some grew only one. More foraminifera survived than expected. Low survival was expected because calcein staining stresses foraminifera and often 90% die if kept in calcein longer than six weeks (Kurtarkar et al., 2015). During the course of this study, the test subjects did not reproduce. While they have reproduced in other studies (Hintz et al., 2004; Mewes et al., 2014), we still did not expect them to reproduce due to environmental stressors (e.g. calcein staining, unfavorable temperatures, unfavorable seawater Mg/Ca, etc.) (Bradshaw, 1957; Kurtarkar, 2015).

The foraminifera observed in this study changed their shell chemistry gradually (each chamber being slightly higher/lower in Mg/Ca than the previous when placed in seawater that is higher/lower than natural seawater, respectively). Chambers followed a trend of increasing or decreasing Mg/Ca rather than an immediate change to calcite reflecting the artificial seawater in which they were immersed. This suggests that peneroplids have a biological mechanism for storing biomineralization materials (in this case, divalent cations) for later use in construction of their test (Bentov and Erez, 2006).

Foraminifera have two ways to biomineralize: one, being directly from the parent seawater, and two, using their own cell-derived fluids (Bentov and Erez, 2006). The advantage of using cell-derived fluids is that the cell predominantly dictates the composition of the calcifying solution, however, the foraminifera has to expend a great amount of energy to do this (Bentov and Erez, 2006). In high-Mg calcite Milliolid foraminifera that use cell-derived fluids to create their tests, such as *P. planatus*, microcrystalline calcite is precipitated within cellular vesicles and is assembled at the chamber formation site (Bentov and Erez, 2006). This explains the inter-chamber and intra-chamber variations seen. Therefore, the gradual change of Mg/Ca ratio in the foraminiferal test when placed in experimental seawater is expected.

3.6.3 Biomineralization below Mg/Ca = 1 and below 25°C

Past research has shown that that increased seawater Mg/Ca drives increases in the Mg/Ca of biotic calcite (Ries, 2004; Hasiuk and Lohmann, 2010). This was the general trend in our 25°C temperature set (Figure 14). However, our 12.5°C temperature set yielded the opposite relationship. The foraminifera that grew at 12.5°C (Figure 15) had three different water chemistries (0.6, 0.8, and 1.0) to use as data points.

As previously stated, aragonite precipitated from seawaters with a Mg/Ca ratio of 0.6 and 0.8. If these inorganic precipitates had altered the chemistry of the growth solution, they would have pushed Mg/Ca ratios higher by removing Ca from the solution. Higher solution Mg/Ca should have been reflected in higher test Mg/Ca. Lower Mg/Ca in marine calcite has been documented as a function of lower temperature (Morse et al., 1997), but it has been shown to be a subordinate factor with respect to solution Mg/Ca (Fuchtbauer and Hardie, 1980). At Mg/Ca ratio of 0.6, the highest foraminiferal Mg/Ca levels occur at the lowest temperature and the lowest foraminiferal Mg/Ca levels occur at the highest temperature (Figure 18). This is likely due to the precipitation of aragonite in the water; however, we noticed the trend of colder jars precipitating more aragonite. This would explain why the foraminifera with the highest Mg levels came from the lowest temperature set: because more Ca was precipitated out at lower temperatures, thereby increasing the Mg/Ca ratio of the seawater. At 12.5°C, aragonite precipitation likely altered the seawater for seawater with Mg/Ca of 0.6 and 0.8 (jars where aragonite precipitation was very noticeable). This is also possible for the 25°C temperature set (Figure 14), but conclusions should be considered tentative due to the paucity of data points beyond the second chamber for subject that grew in Mg/Ca = 1.0.

The 3°C specimens (Figure 16) again showed higher than expected test Mg/Ca for the effect of seawater with Mg/Ca ratio of 0.6. This is likely due to aragonite precipitation. It is possible that a large enough amount precipitated that significantly altered the artificial seawater Mg/Ca so that the seawater that despite beginning at 0.6 or 0.8 rose it rose to higher levels.

3.7 Conclusions

3.7.1 Summary

Benthic foraminifera serve as an accurate indicator of global ocean temperatures, because they inhabit deep-ocean water masses less affected by high-frequency variations in sea surface temperature and salinity and are therefore ideal for culture studies. The response of foraminiferal calcite to both seawater Mg/Ca and temperature was tested in the laboratory through a set of culture experiments that varied seawater Mg/Ca and temperature. *Peneroplis planatus* was grown for 41 days at 25°C in artificial seawater with Mg/Ca ranging from 0.6 to 8.0 mol/mol. Surviving specimens were analyzed via electron microprobe.

The high-Mg benthic foraminifer, *Peneroplis planatus*, was cultured in the laboratory at a variety of water temperatures and water Mg/Ca. After 41 days the foraminifera were analyzed by electron microprobe. Data from foraminifera grown at 25°C and Mg/Ca between 1.0 and 8.0 display a power law relationship between water Mg/Ca and test Mg/Ca, as measured on the 3rd chamber of new growth. This is only the second such calibration produced for high-Mg foraminifera. Data from foraminifera grown below 25°C and below Mg/Ca = 1 were inconclusive and will need to be re-run before a complete calibration for the effects of seawater temperature and Mg/Ca can be generated.

Having completed this study, the following research questions remain to be addressed:

- ***How does foraminiferal Mg/Ca respond to seawater temperatures below 25°C?*** More experimentation needs to be done to retrieve data for foraminifera cultured in seawater with Mg/Ca above 1.0 but in temperatures lower than 25°C. Our results of seawater with a Mg/Ca below 1.0 were not reliable, due to aragonite precipitation. It is expected that foraminiferal Mg/Ca will increase with increasing seawater Mg/Ca, and not the inverse relationship, as we saw in our limited data. The same experimental process can be done using a larger number of foraminifera to assure a larger amount of data in order to have a large enough amount of data to draw conclusions. With this new temperature and Mg/Ca data, a temperature regression can also be done.
- ***How does foraminiferal Mg/Ca respond to seawater Mg/Ca lower than 1.0?*** It is expected that with decreasing seawater Mg/Ca, foraminiferal Mg/Ca would decrease. This same experimental method can be used, however, seawater should be bubbled with CO_2 to lower the pH to keep the aragonite from precipitating.
- ***How does the Mg/Ca in low-Mg calcite foraminifera respond to changes in seawater Mg/Ca and temperature?*** Similar results to our high-Mg calcite foraminifera would be expected in that, higher seawater Mg/Ca and temperatures would produce foraminifera

with higher test Mg/Ca. However, this effect may not be as pronounced in low-Mg calcite foraminifera. This same experimental process can be conducted. A larger number of foraminifera should be used (at least 4,000), however, due to previous experiments indicating that low-Mg calcite foraminifera prefer lower Mg/Ca ratios. It is likely that higher seawater Mg/Ca ratios stress low-Mg calcite foraminifera. A greater number is needed to ensure enough data can be obtained.

- ***How long do foraminifera need to be grown in experimental conditions for chamber Mg/Ca to stabilize?***
  In this study, the Mg/Ca of the 3rd new chamber was used to derive an equation (Equation 2) that describes how fluid Mg/Ca affects foraminifer Mg/Ca. None of the foraminifera, however, showed two successive chambers of constant Mg/Ca suggesting that they may not have reached equilibrium with fluid Mg/Ca. It would be useful to identify how many chambers are needed to reach equilibrium as it would indicate how large a store of ions a foraminifer possesses. It is estimated that equilibrium should be attained by the time ten new chambers (approximately 90 days) have grown.

3.8 FIGURES

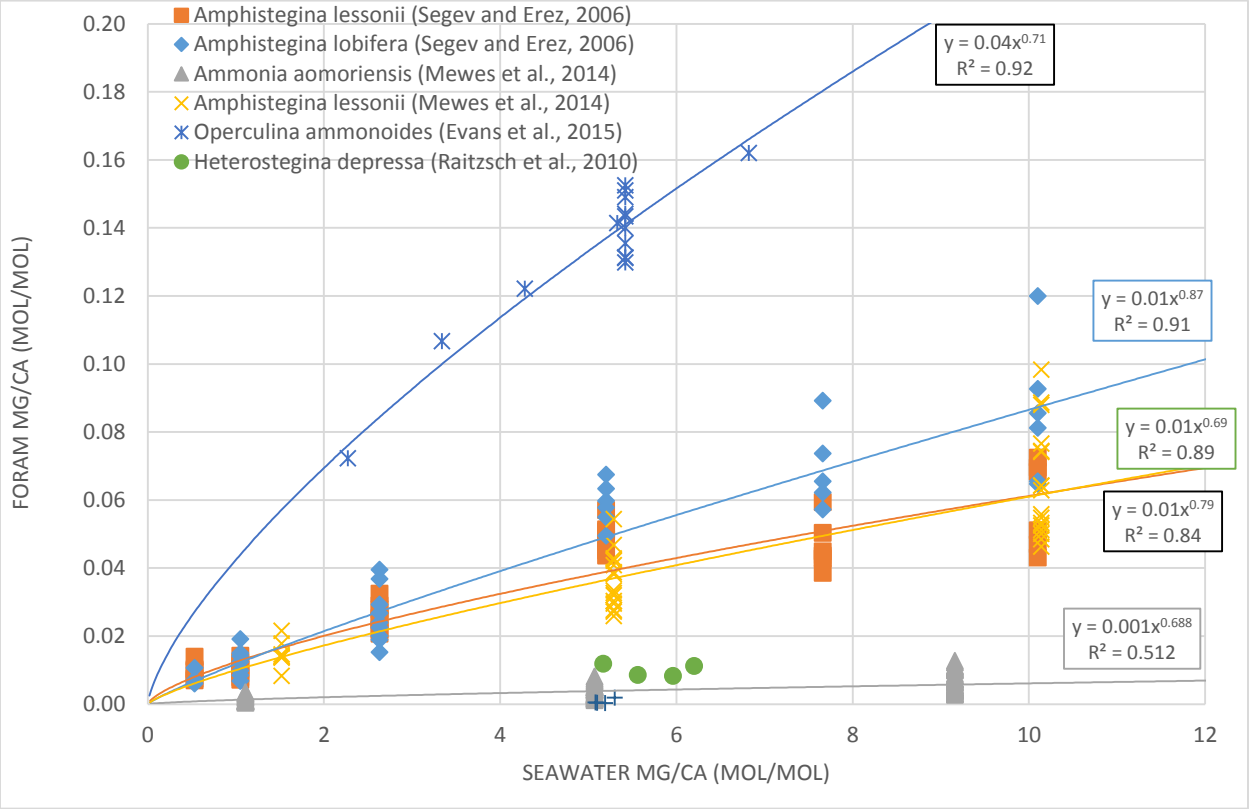

Figure 9: Research on foraminifera culturing in seawater with different Mg/Ca up to present. The blue diamond, orange square, and yellow cross markers indicate intermediate Mg-calcite species. The green dot, blue cross, and gray diamond markers indicate a low-Mg calcite species. The blue stars indicate high-Mg calcite.

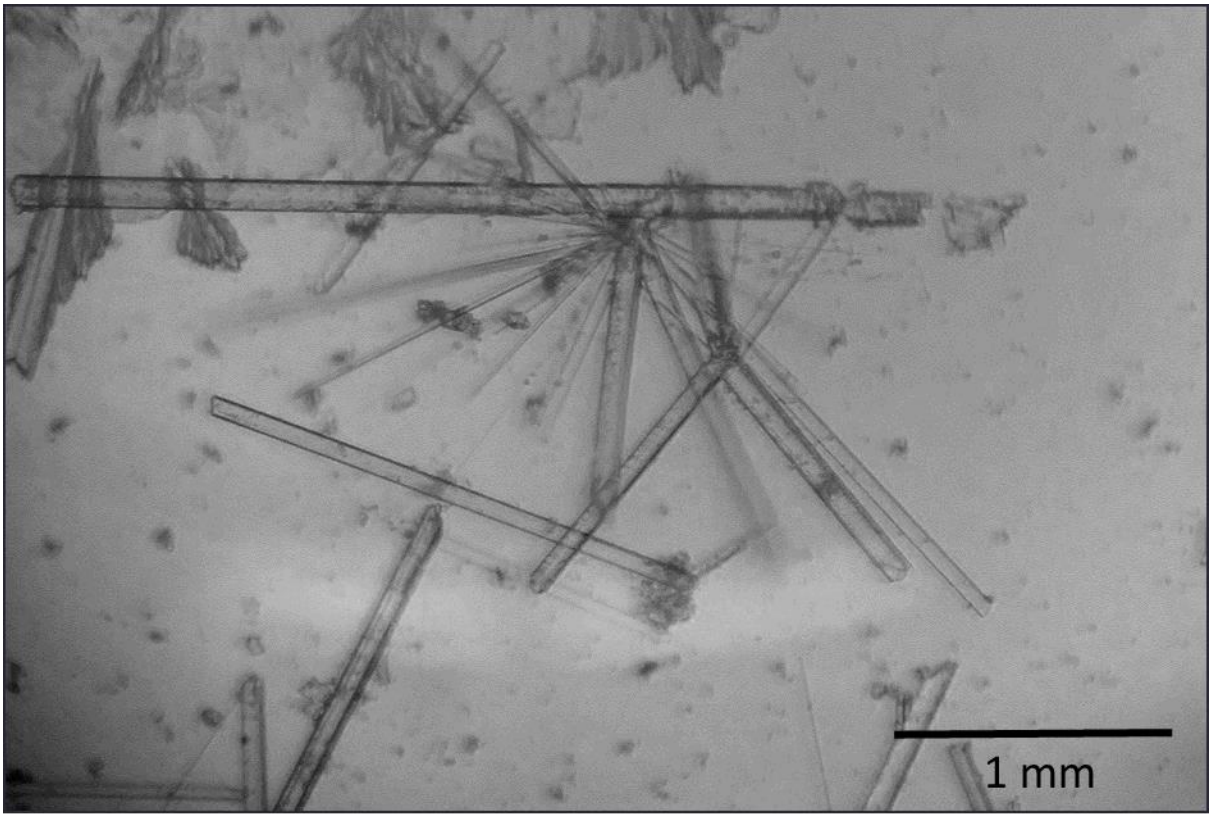

Figure 10: Photograph of aragonite crystals that precipitated in experimental cultures with Mg/Ca < 1.0 mol/mol.

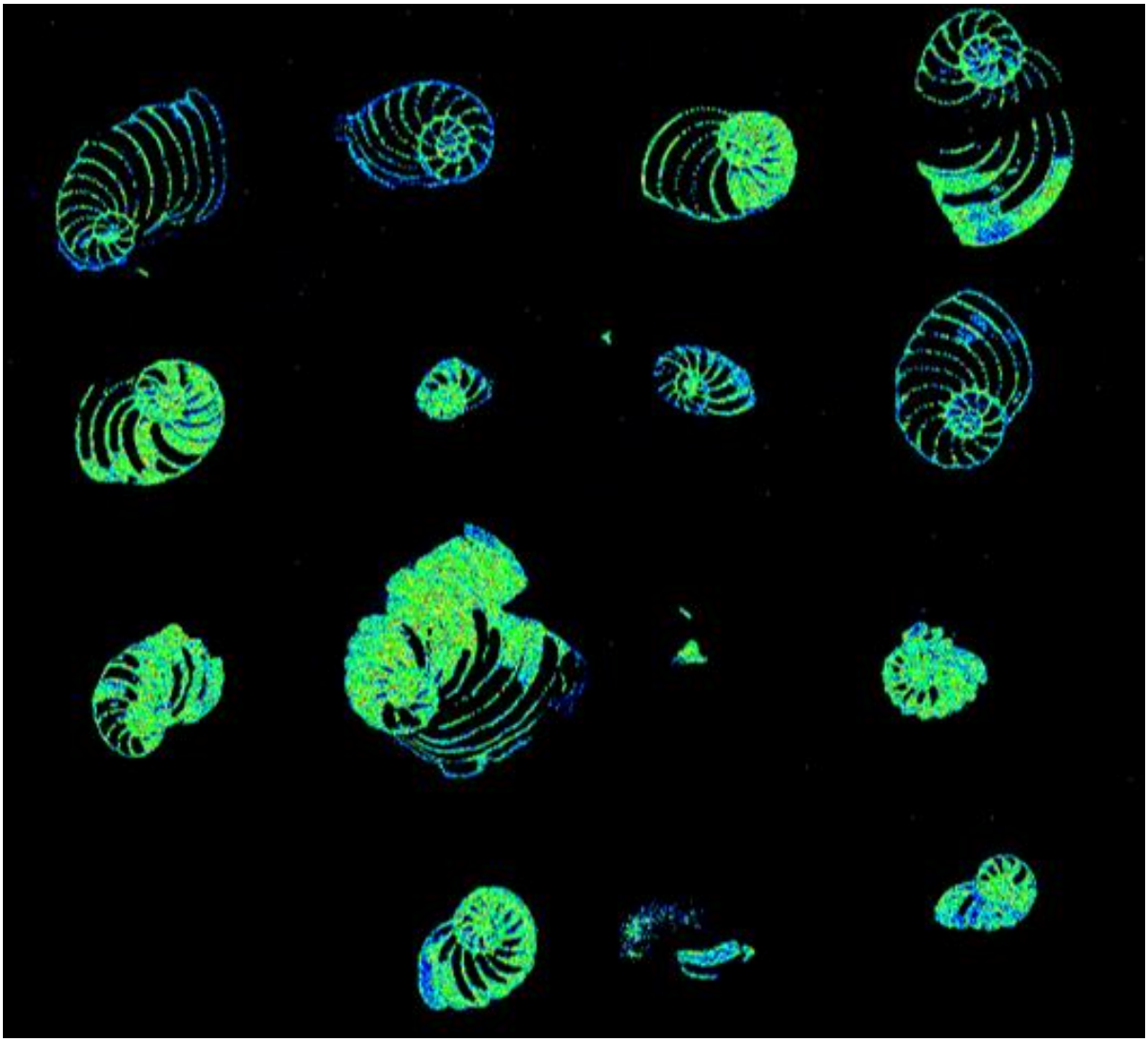

Figure 11: Qualitative elemental maps of Ca in 20 foraminifera grown under experimental conditions (some are missing due to damage incurred during polishing). Brighter colors indicate more calcium (red being the highest), while cooler colors indicate less calcium (blue being the lowest).

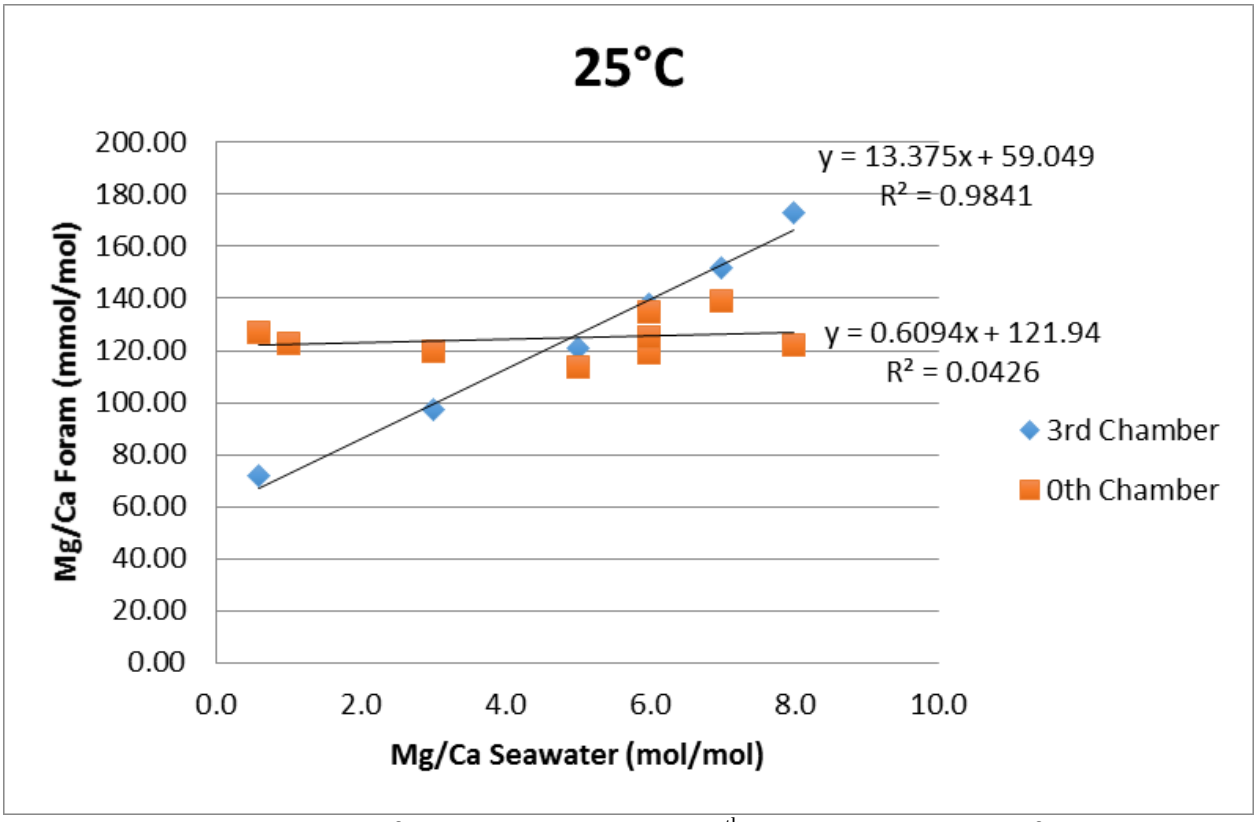

Figure 12: Linear regression of the 25° C data set. The 0th Chamber is the start of experimental growth. Error bars were too small to be shown, as they are contained within the data points.

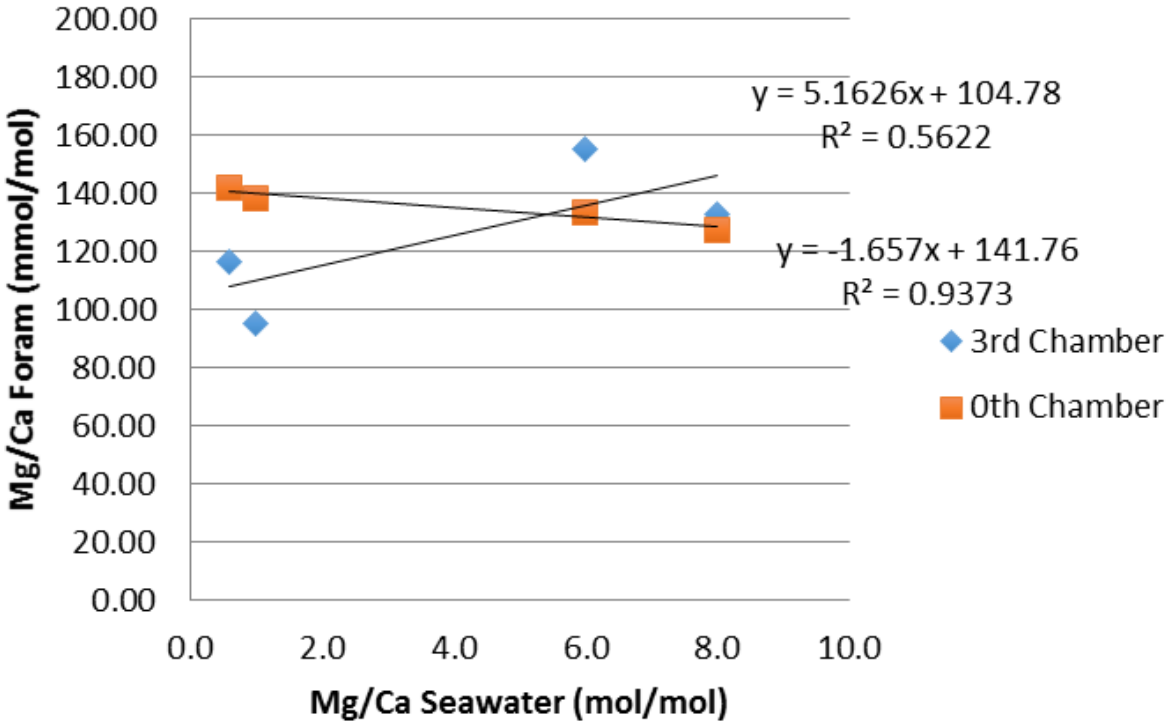

Figure 13: Linear regression of the 3°C data set. The 0th Chamber is the start of experimental growth. Error bars are contained within the data points.

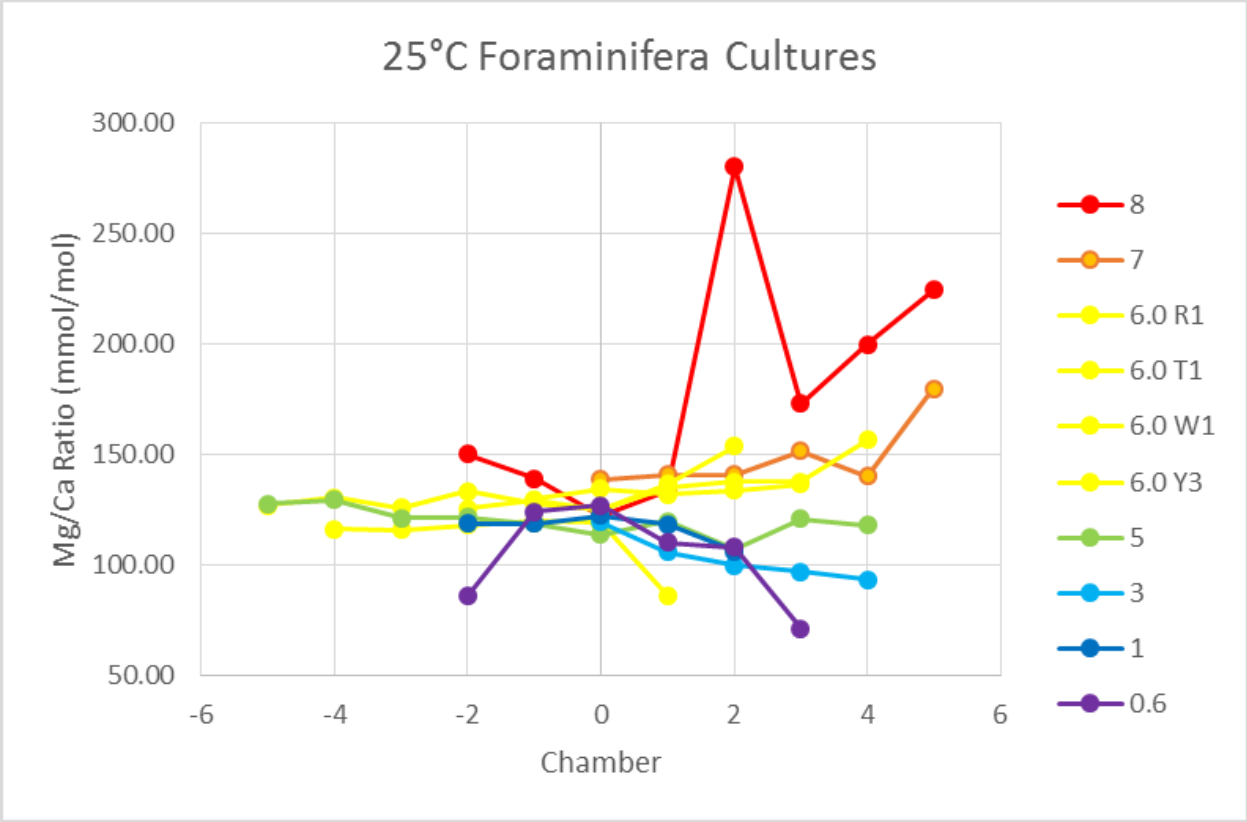

Figure 14: Graph of 25°C temperature set for all seawater Mg/Ca obtained (listed at right). Warmer colors indicate higher seawater Mg/Ca. The sharp peak in the Seawater Mg/Ca 8.0 up to approximately 275 mmol/mol is believed to be an outlier. Error bars were too small to be shown, as they are contained within the data points. Temperature ranged from 23.01-25.21°C for the 25°C incubator over the length of the experiment.

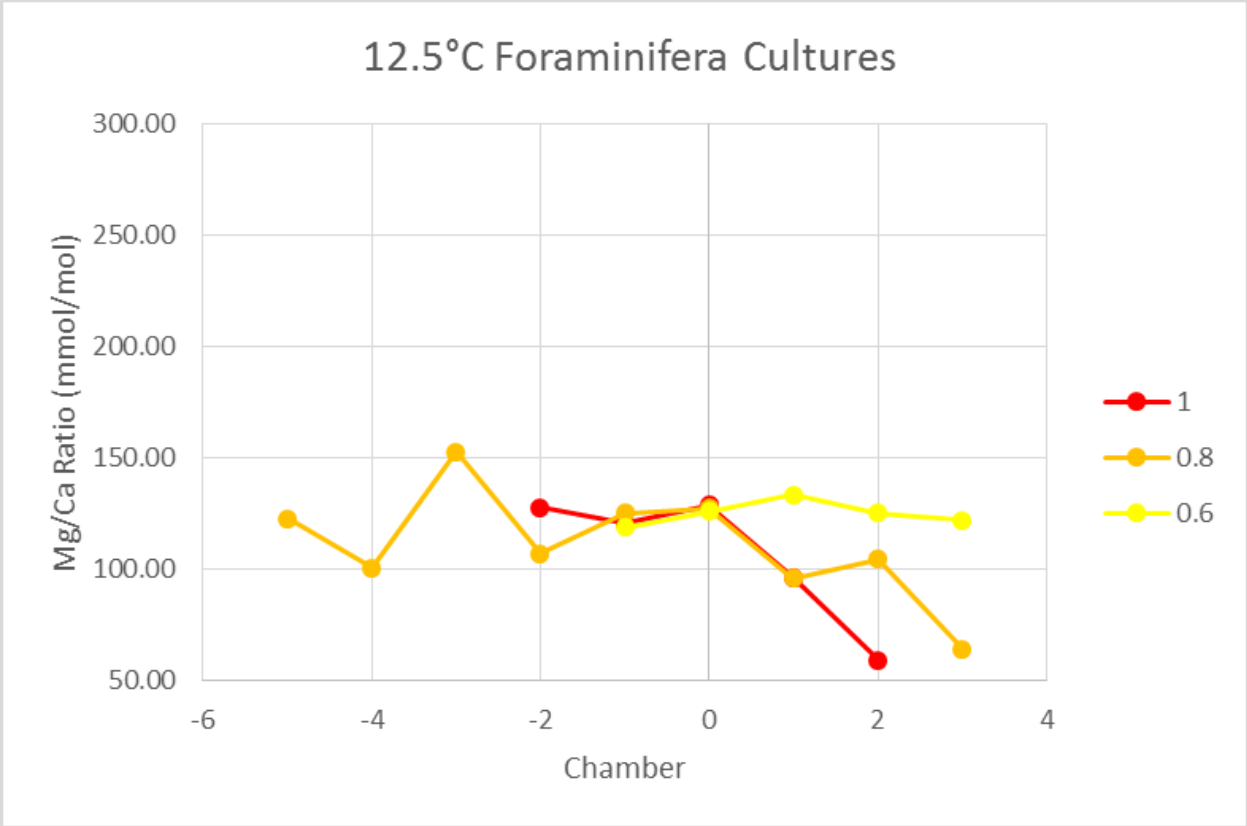

Figure 15: Graph of 12.5°C temperature set for all seawater Mg/Ca obtained (listed at right). Warmer colors indicate higher seawater Mg/Ca. Error bars were too small to be shown, as they are contained within the data points. Temperature ranged from 13.43-16.13°C for the 12.5°C incubator over the length of the experiment.

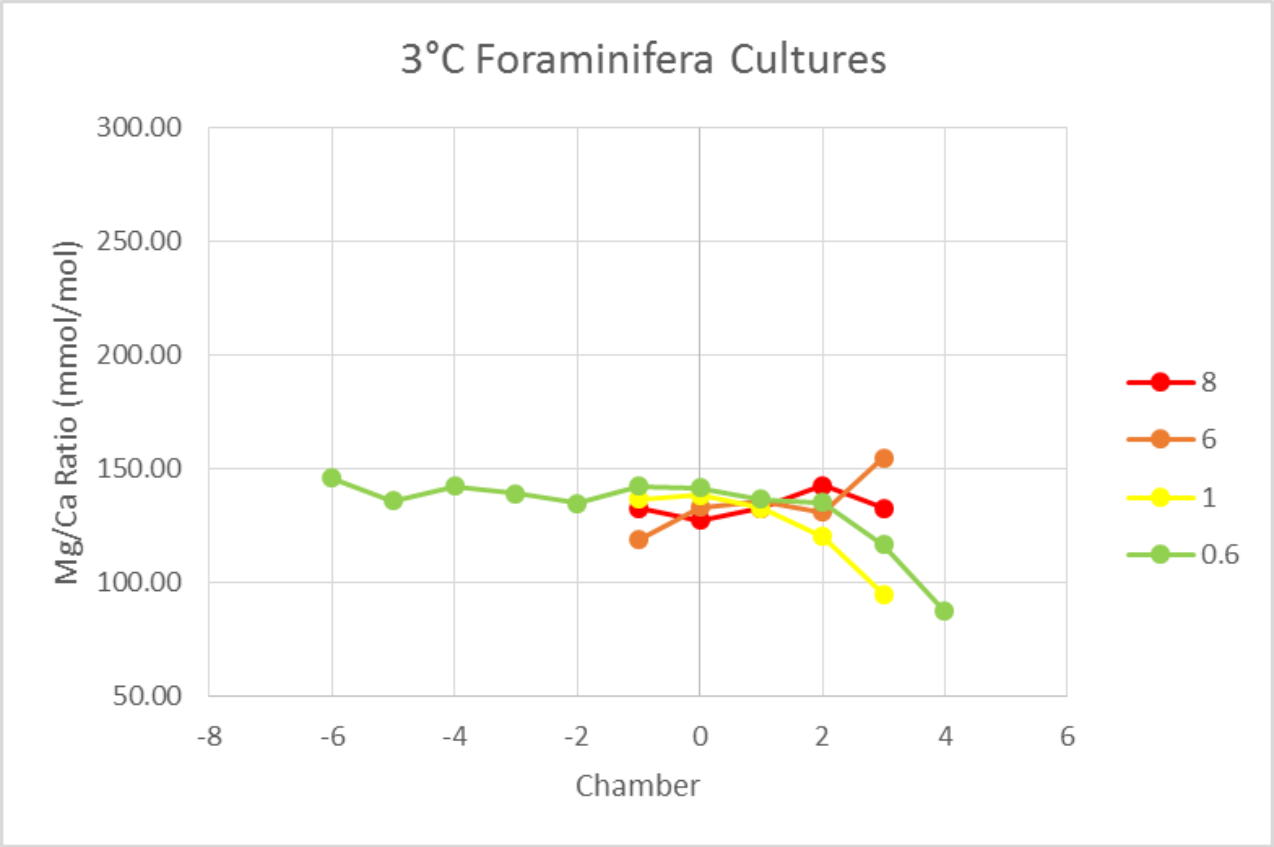

Figure 16: Graph of 3°C temperature set for all seawater Mg/Ca obtained (listed at right). Warmer colors indicate higher seawater Mg/Ca. Error bars were too small to be shown, as they are contained within the data points. Temperature ranged from 3.04-3.99°C for the 3°C incubator over the length of the experiment.

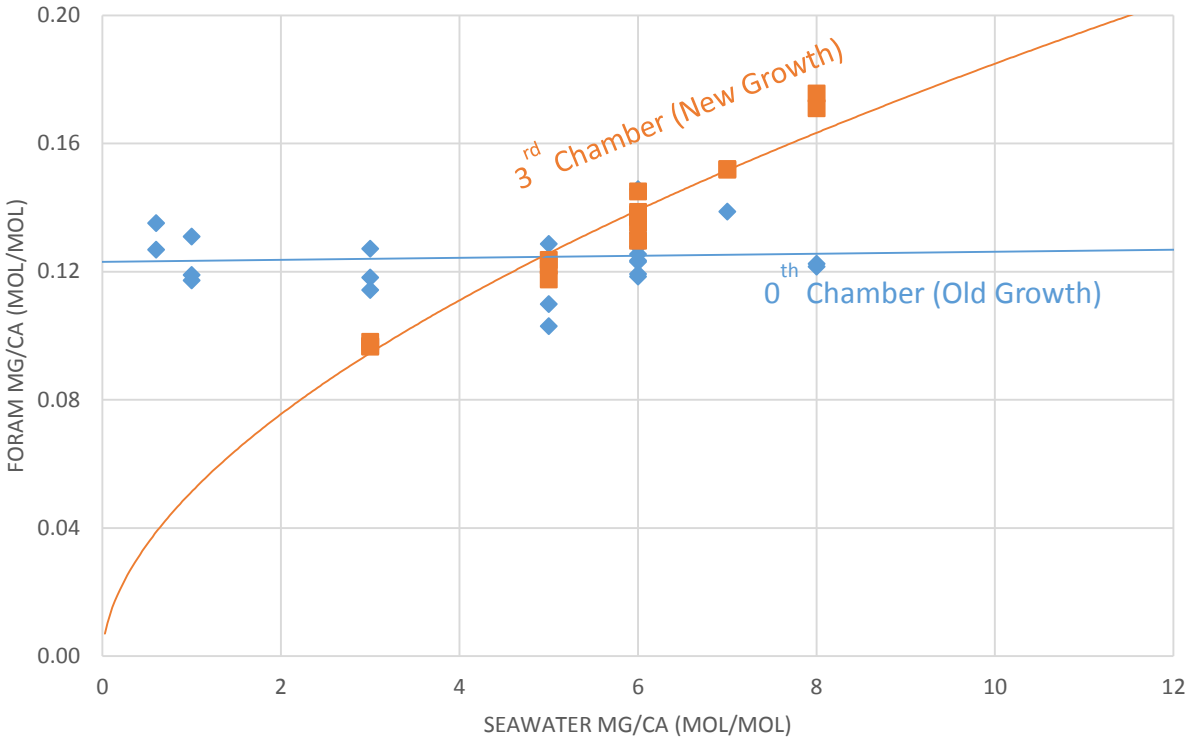

Figure 17: Foraminifer Mg/Ca plotted against seawater Mg/Ca for the 0th chamber of growth and the 3rd chamber of growth. The 0th chamber of growth is the start of experimental conditions, prior to any experimental influence. New growth is different from old growth.

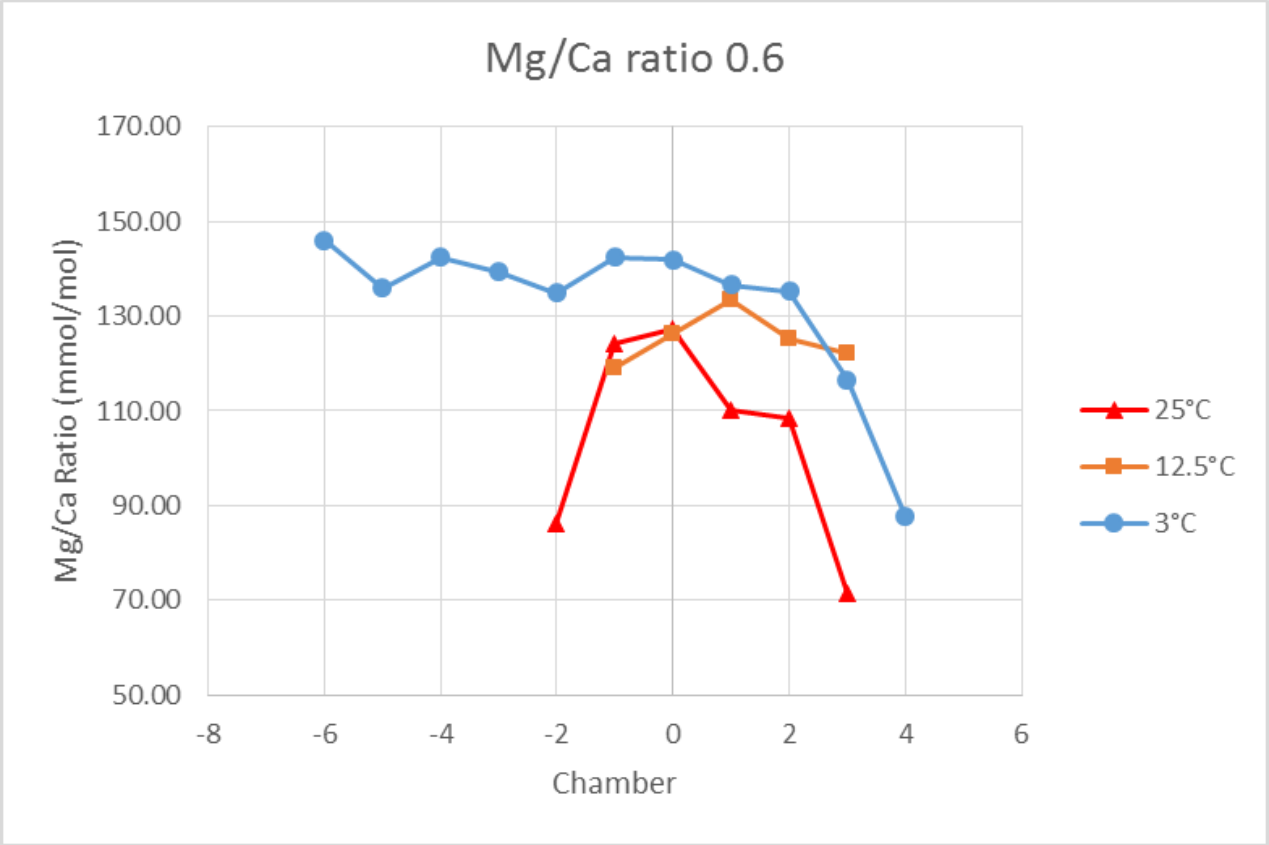

Figure 18: Seawater Mg/Ca ratio of 0.6 showing foraminiferal response in three different temperature sets. Error bars were too small to be shown, as they are contained within the data points.

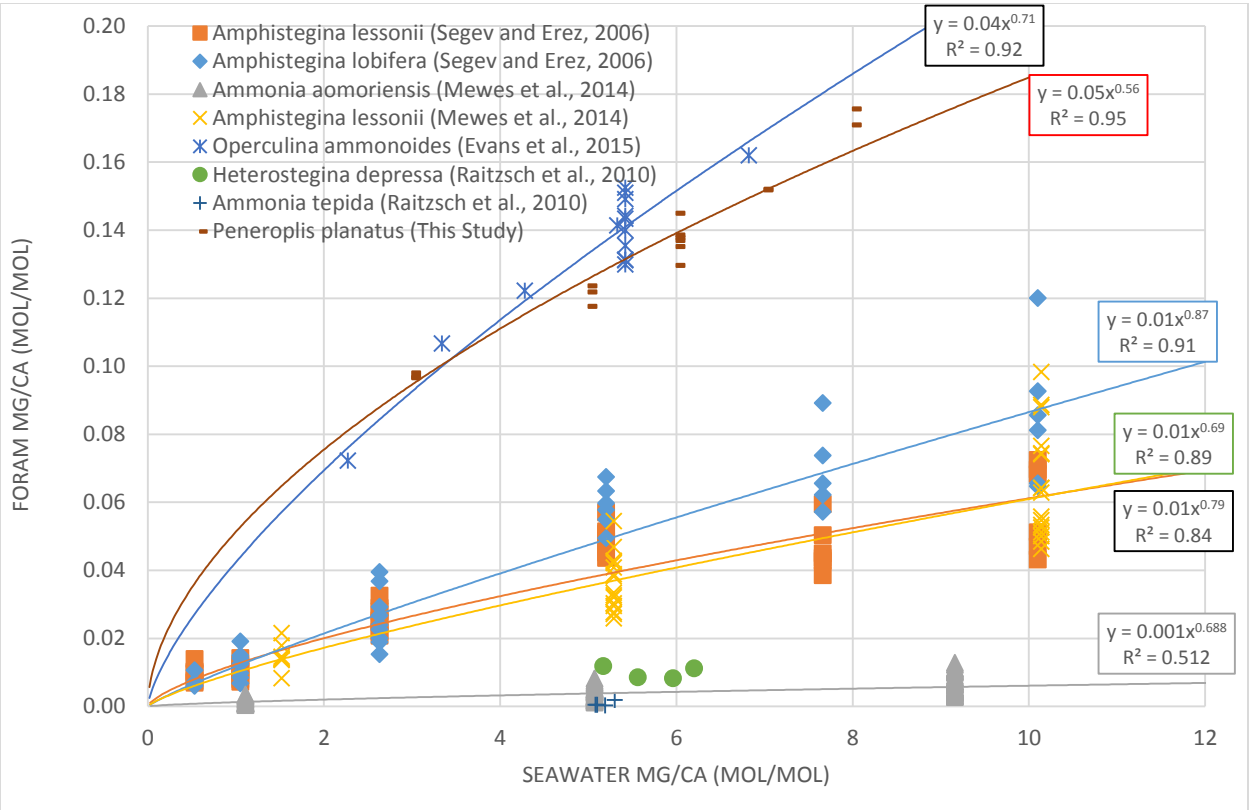

Figure 19: Research on foraminifera culturing in seawater with different Mg/Ca up to present with data from this study plotted. The blue diamond, orange square, and yellow cross markers indicate intermediate Mg-calcite species. The green dot, blue cross, and gray diamond markers indicate a low-Mg calcite species. The blue stars and the red dashes indicate high-Mg calcite. The blue stars show data from Evans et al, 2015, the only other comparable study. The red dashes show data from this study. Fewer correlations can be drawn from low-Mg calcite species due to a very small range of seawater Mg/Ca experimented with.

3.9 TABLES

Table 1. Seawater Mg/Ca and Temperature conditions for peneroplid jar experiment, “CAL” indicates aragonite precipitated in the jar, “G” indicates that at least one foraminifera grew in the jar during the experiment, “G+ARG” indicates that aragonite precipitated and at least one foraminifera grew in the jar during the experiment

		Culture Temperature		
		3.04-3.99°C	**13.43-16.13°C**	**23.01-25.21°C**
Seawater Mg/Ca	**0.6**	G+ARAG	G+ARAG	G+ARAG
	0.8	G+ARAG	G+ARAG	G+ARAG
	1.0	G	G	G
	2.0	G	G	G
	3.0	G	G	G
	4.0	G	G	G
	5.0	G	G	G
	6.0	G	G	G
	7.0	G	G	G
	8.0	G	G	G

Table 2. Summary of specific point mapped foraminifera, their seawater Mg/Ca, T, number of new chambers, and number of specific data points taken.

Foraminifer Id	Temperature	Seawater Mg/Ca	Number of New Chambers Grown	N
S1	25	0.6	4	14
M1	25	1.0	3	15
C1	25	3.0	4	15
Aa1	25	5.0	4	27
R1	25	6.0	4	36
T1	25	6.0	3	11
W1	25	6.0	3	10
Y3	25	6.0	3	15
Q1	25	7.0	5	7
P1	25	8.0	5	12
Aa2	12.5	0.6	3	15
Bb2	12.5	0.8	3	21
N1	12.5	1.0	2	15
Cc1	3.0	0.6	4	18
O2	3.0	1.0	3	15
Z1	3.0	6.0	3	15
X1	3.0	8.0	3	16

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CHAPTER IV. CONCLUSIONS

4.1 Chapter II: A RECIRCULATING SYSTEM TO SUPPORT A COLONY OF BENTHIC FORAMINIFERA

The objectives of this study were to construct a foraminiferal culture system that could provide specimens for various experiments that seek to measure the relationship between foraminifera Mg/Ca and seawater Mg/Ca and temperature. Though only a single cell, foraminiferal tests have been shown to record several environmental parameters (i.e. source water composition, temperature, etc.). A majority of the culturing studies on foraminifera have introduced foraminifera directly into experimental conditions. However, for those far from the ocean, and those who wish to maintain multiple species from various locations around the world, this proves difficult, time-consuming, and expensive. A foraminiferal culture system was designed after a thorough review of the literature and in consultation with local aquaculture, biology, and engineering experts to maintain foraminifera indefinitely. These foraminifera were used to conduct experiments that measured the relationship between seawater Mg/Ca and foraminiferal Mg/Ca. With these experiments, measuring the relationship between seawater temperature and foraminiferal Mg/Ca was attempted.

Previous aquaculture systems have been used for future research studies; this system presented here was partially modeled after those systems (Hintz et al., 2004; Ries, 2004). Foraminiferal culture experiments are usually conducted over short time intervals (three months), with test subjects being replenished from ocean sources for each experiment. However, in order to conduct multiple culturing studies far from a source of new test subjects, a long-term system was needed to maintain active foraminiferal cultures. Such a “colony” has not been described in the literature recently (e.g. Zillioux, 1969).

The foraminiferal culture system constructed for this study is a long-term system that can be maintained and used indefinitely. This inexpensive and easy to construct system allows for several organisms to be maintained in culture at once time, allowing for various research avenues to be explored (i.e. foraminifera studies, species interaction research, vertebrate research, etc.). The large filtration subsystem allows each individual colony reservoir to contain its own species set without contaminating other colony reservoirs. The filtration subsystem also removes the need for sealed colony reservoirs employed by many researchers looking to reduce contamination. Unsealed colony reservoirs allow easier access to system components and specimens. Additionally, outside of the realm of academia, systems like these have proven useful, robust, and easy to maintain among hobbyist aquaculture. School classrooms can also use this system for hands-on scientific learning.

4.2 Chapter III: GROWTH OF FORAMINIFERA IN SEAWATER WITH VARYING WATER MG/CA AND TEMPERATURES TO AID IN CALIBRATION OF THE BENTHIC FORAMINIFERAL CENOZOIC PALEOCRYOMETER

High-Mg benthic foraminifera grown in laboratory cultures were to assess how the Mg/Ca and temperature of seawater affect the Mg/Ca in a foraminiferal test. When this relationship can be determined, it can be combined with other data (like long-term trends in foraminiferal $\delta^{18}O$ and seawater Mg/Ca) to generate a times series for continental glaciations, global sea level, and climate over deep time (e.g. Lear, 2000). Experiments were conducted that varied both seawater temperature and chemistry. Their subsequent effects on foraminiferal tests were planned to be used to derive a more robust relationship between seawater and test chemistry. The ultimate goal of this research program was to derive a more accurate picture of climate evolution over the last 50 million years. This could be constructed from the wealth of data that exist on foraminiferal $\delta^{18}O$ and seawater Mg/Ca over this time period. The specific goals of this study, again, were to:

1. Use the previously designed culture system to retrieve viable test subjects
2. Mark specimens successfully to be able to determine new growth
3. Successfully grow foraminifera in experimental seawaters with varying Mg/Ca and temperatures
4. Analyze foraminifera for Mg/Ca shell chemistry
5. Assess how foraminiferal Mg/Ca shell chemistry is affected by both temperature and seawater Mg/Ca
6. Derive a function of foraminifera Mg/Ca to derive temperature in order to relate it back to paleo-temperatures
7. Apply this information over the last 50 million years

The experimental system was built and foraminifera were exposed to experimental conditions for 41 days before harvest. Due to the loss of some foraminifera during specimen processing, an incomplete dataset was generated. Linear regressions were done to correlate seawater Mg/Ca to foraminiferal Mg/Ca. While the data can be fit by either a linear or power function with $r^2 > 0.9$, the power function is more widely comparable to other calcifying invertebrates. Temperature was not able to be correlated due to missing specimens, so this data cannot be incorporated into the current analysis. The outcomes of this study support those reported recently (Evans et al., 2015) on the high-Mg benthic foraminifer *Operculina ammonoides*. Having a more complete picture of how foraminiferal shell Mg/Ca is affected by seawater Mg/Ca will have much broader implications in paleo-climatological analysis by helping calibrate the benthic foraminiferal $\delta^{18}O$ paleo-cryometer.

4.3 References

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APPENDICES

APPENDIX A. SEAWATER RECIPE USED FOR PENEROPLID JAR EXPERIMENT

	Target Mg/Ca (mol/mol)									
	0.6	**0.8**	**1.0**	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**	**7.0**	**8.0**
	Grams of salt per 3 L									
NaCl	~72	~72	~72	~72	~72	~72	~72	~72	~72	~72
Na_2SO_4	12.024	12.024	12.024	12.024	12.024	12.024	12.024	12.024	12.024	12.024
KCl	2.031	2.031	2.031	2.031	2.031	2.031	2.031	2.031	2.031	2.031
$NaHCO_3$	0.588	0.588	0.588	0.588	0.588	0.588	0.588	0.588	0.588	0.588
KBr	0.294	0.294	0.294	0.294	0.294	0.294	0.294	0.294	0.294	0.294
H_3BO_3	0.078	0.078	0.078	0.078	0.078	0.078	0.078	0.078	0.078	0.078
NaF	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009
$SrCl_2•6H_2O$	0.072	0.072	0.072	0.072	0.072	0.072	0.072	0.072	0.072	0.072
$MgCl_2•6H_2O$	**33.739**	**33.739**	**33.739**	**33.739**	**33.739**	**33.739**	**33.739**	**33.739**	**33.739**	**33.739**
$CaCl_2•2H_2O$	**39.750**	**29.800**	**23.852**	**11.922**	**7.949**	**5.961**	**4.769**	**3.974**	**3.406**	**2.980**
H_2O	2845	2853	2858	2868	2875	2874	2875	2875	2876	2876
Mg (mol/kg)	0.056	0.056	0.056	0.056	0.056	0.056	0.056	0.056	0.056	0.056
Ca (mol/kg)	0.093	0.069	0.056	0.028	0.019	0.014	0.011	0.009	0.008	0.007
Mg (ppm)	1350	1350	1350	1350	1350	1350	1350	1350	1350	1350
Ca (ppm)	3710	2781	2226	1113	742	556	445	371	318	278
TOTAL MOLES	0.148	0.125	0.111	0.083	0.074	0.069	0.067	0.065	0.063	0.062

Methods

Seawater was mixed in batches of 3L in a multistep process. First, gravimetric salts (salts that are not hydrated) were dried at 150ºC for approximately two weeks prior to mixing. All ingredients except hydrated salts were then weighed and added to 2L of water. Hydrated salts were weighed and added to the amount of remaining water in a separate container. Hydrated salt solution was added to the gravimetric salt solution and mixed. Salinity was 30ppt for every mixture.

APPENDIX B. QUANTATIVE GEOCHEMICAL DATA FROM PENEROPLID JAR EXPERIMENT (CHAPTER III)

All atom ratio data (in mmol/mol) presented here were obtained from foraminifera via electron microprobe with a 5-micron spot size. Chamber refers to the foraminiferal chamber, with positive numbers being new growth, zero being the start of the experimental conditions, and negative numbers signifying growth that occurred prior to experimental conditions. The "I/C/O" column refers to the location on the foraminifer that the data was measured: "O" refers to the "outside" of the foraminiferal chamber, where the new growth is not attaching to any coiled old growth; "I" refers to the "inside" of the foraminifer, which is the area of new growth that attaches to old, coiled growth area; "C" refers to the "center" of the foraminifer's chamber, which is situated between "I" and "O". n= 276.

Data point	Chamber	I/C/O	Mg/Ca	Sr/Ca	Fe/Ca	Mn/Ca	Ba/Ca
			(mmol/mol)				
Plug1_AA1_1	4	I	101.1	1.7	0.3	0.3	0.0
Plug1_AA1_2	3	I	117.6	2.8	0.6	0.3	-0.9
Plug1_AA1_3	2	I	111.0	2.9	0.3	0.1	0.2
Plug1_AA1_4	1	I	125.3	3.3	-0.1	0.2	0.2
Plug1_AA1_5	0	I	128.7	1.8	0.6	-0.9	-0.9
Plug1_AA1_6	-2	I	120.3	1.8	-0.4	-2.2	-0.8
Plug1_AA1_7	-2	C	118.0	1.8	0.2	0.1	-0.1
Plug1_AA1_8	-1	C	114.5	2.9	0.0	-0.7	-1.5
Plug1_AA1_9	0	C	103.0	2.3	-0.5	0.4	0.0
Plug1_AA1_10	1	C	118.3	2.5	0.7	-0.4	0.2
Plug1_AA1_11	2	C	111.1	2.3	0.6	-0.1	0.0
Plug1_AA1_12	3	C	121.8	2.8	0.4	-0.5	-1.1
Plug1_AA1_13	4	C	111.9	2.3	0.1	0.3	-0.2
Plug1_AA1_14	4	O	133.3	2.3	0.5	1.3	0.2
Plug1_AA1_15	3	O	123.7	2.7	0.1	0.1	0.1
Plug1_AA1_16	2	O	100.4	3.1	-0.4	0.2	0.0
Plug1_AA1_17	1	O	116.6	2.4	0.0	-2.6	-0.8
Plug1_AA1_18	0	O	109.9	2.4	0.5	0.2	-0.5
Plug1_AA1_19	-1	O	123.1	2.0	-0.1	-1.8	-0.8
Plug1_AA1_20	-2	O	127.0	2.0	0.1	0.2	0.0
Plug1_AA1_21	-3	I	121.6	2.5	-0.2	-1.8	-1.4
Plug1_AA1_22	-4	I	129.6	2.2	0.7	-1.6	-0.6
Plug1_AA1_23	-5	I	127.9	2.6	-0.2	-0.7	-0.5
Plug1_AA1_24	-6	I	122.5	2.4	0.3	-2.2	-0.5

Plug1_AA1_25	-7	I	135.9	1.9	0.0	-3.5	-1.3
Plug1_AA1_26	-8	I	128.9	2.5	0.2	-0.5	-0.2
Plug1_AA1_27	-9	I	131.0	2.9	0.5	-1.6	-0.5
Plug1_AA2_1	3	I	119.9	2.0	0.7	-2.1	-0.8
Plug1_AA2_2	2	I	116.2	2.2	0.8	0.5	0.1
Plug1_AA2_3	1	I	130.9	2.7	0.2	-2.2	-0.7
Plug1_AA2_4	0	I	129.4	2.2	0.3	-1.6	-1.6
Plug1_AA2_5	-1	I	125.2	1.7	0.1	-0.7	-0.7
Plug1_AA2_6	-1	C	118.5	2.3	0.7	-0.5	-0.3
Plug1_AA2_7	0	C	125.7	2.0	-0.1	-1.1	-0.7
Plug1_AA2_8	1	C	140.2	2.5	0.4	0.2	0.0
Plug1_AA2_10	3	C	119.8	2.4	-0.1	0.1	0.0
Plug1_AA2_11	2	C	125.1	3.3	0.1	-1.0	-0.4
Plug1_AA2_12	3	O	126.9	2.6	0.4	-0.3	-0.8
Plug1_AA2_13	2	O	134.3	2.3	0.5	0.0	0.1
Plug1_AA2_14	1	O	129.5	2.0	0.2	0.4	-0.4
Plug1_AA2_15	0	O	123.4	2.3	-0.3	-1.3	-0.9
Plug1_AA2_16	-1	O	113.1	2.7	0.1	0.0	0.1
Plug1_CC1_1	4	I	87.6	1.8	-0.2	0.2	0.0
Plug1_CC1_2	3	I	94.4	1.7	-0.3	1.2	0.3
Plug1_CC1_3	2	I	128.9	2.5	-0.4	-0.4	0.0
Plug1_CC1_4	1	I	129.0	1.7	0.3	0.2	0.1
Plug1_CC1_5	0	I	134.7	2.0	-0.3	0.1	-0.1
Plug1_CC1_6	-1	I	143.0	1.9	0.1	0.0	0.0
Plug1_CC1_7	-2	I	143.6	1.5	0.4	0.5	0.1
Plug1_CC1_8	-3	I	138.3	2.0	-0.3	0.2	-0.1
Plug1_CC1_9	-4	I	139.4	3.0	-0.2	-0.7	-0.5
Plug1_CC1_10	-5	I	142.4	2.5	0.5	-2.3	-1.7
Plug1_CC1_11	-6	I	135.9	2.2	0.2	0.5	0.3
Plug1_CC1_12	-7	I	146.1	1.8	-0.1	-4.5	-0.9
Plug1_CC1_13	-3	C	131.4	1.9	0.2	-1.7	-0.6
Plug1_CC1_14	-2	C	141.3	2.0	0.5	-1.3	-1.1
Plug1_CC1_15	-1	C	141.9	1.7	0.3	-1.2	-0.9
Plug1_CC1_16	1	C	146.2	1.6	0.2	0.1	0.0
Plug1_CC1_17	2	C	141.5	2.4	0.4	-1.9	-1.1
Plug1_CC1_18	3	C	138.9	2.4	0.0	-0.9	-0.9
Plug1_C1_1	4	I	96.9	1.4	0.1	1.5	0.2
Plug1_C1_2	3	I	96.7	1.1	0.6	1.4	0.2
Plug1_C1_3	2	I	96.8	-0.2	-0.2	0.3	0.4

Plug1_C1_4	1	I	106.6	1.5	0.5	-1.3	-0.7
Plug1_C1_5	0	I	118.1	3.0	0.0	-0.1	0.3
Plug1_C1_6	0	C	114.3	1.8	0.0	-1.6	-0.4
Plug1_C1_7	1	C	113.6	1.7	0.1	-1.3	-0.7
Plug1_C1_8	2	C	109.9	0.9	0.6	1.1	1.0
Plug1_C1_9	3	C	98.1	1.4	-0.5	0.5	0.3
Plug1_C1_10	4	C	91.6	0.8	-0.6	1.1	0.3
Plug1_C1_11	4	C	92.0	1.0	-0.5	2.3	0.2
Plug1_C1_12	3	C	96.9	1.1	0.3	1.8	0.4
Plug1_C1_13	2	C	93.6	1.3	0.0	1.0	-0.1
Plug1_C1_14	1	C	98.2	0.9	0.3	0.4	0.2
Plug1_C1_15	0	C	127.1	2.5	-0.2	-2.0	-1.4
Plug3_M1_1	2	I	98.5	1.7	0.1	0.7	0.2
Plug3_M1_2	1	I	132.5	2.1	0.4	-1.0	-1.3
Plug3_M1_3	0	I	131.0	2.0	0.5	0.6	-0.3
Plug3_M1_4	-1	I	129.2	2.3	0.3	-1.0	-1.3
Plug3_M1_5	-2	I	128.0	2.1	-0.3	0.3	0.2
Plug3_M1_6	-2	C	109.1	2.4	-0.2	-0.7	-0.9
Plug3_M1_7	-1	C	116.0	2.2	0.5	-1.3	-0.4
Plug3_M1_8	0	C	119.0	2.0	0.5	-1.4	-0.2
Plug3_M1_9	1	C	118.6	2.1	-0.1	-1.7	-1.2
Plug3_M1_10	2	C	111.2	2.4	0.0	0.3	0.1
Plug3_M1_11	2	O	109.3	2.2	-0.2	0.4	0.0
Plug3_M1_12	1	O	105.2	2.7	0.6	0.6	-0.2
Plug3_M1_13	0	O	117.3	2.8	-0.1	0.6	0.2
Plug3_M1_14	-1	O	111.3	2.7	0.5	0.0	-0.5
Plug3_M1_15	-2	O	119.4	1.8	0.3	-1.3	-0.5
Plug3_N1_1	2	I	68.8	1.6	-0.3	0.4	-0.1
Plug3_N1_2	1	I	112.3	2.4	-0.1	-0.2	0.3
Plug3_N1_3	0	I	131.6	2.3	0.1	-2.1	-1.3
Plug3_N1_4	-1	I	126.4	2.0	0.0	-0.7	-0.1
Plug3_N1_5	-2	I	136.7	2.5	0.2	0.1	-0.2
Plug3_N1_6	-2	M	126.6	2.1	0.3	-0.5	-0.6
Plug3_N1_7	-1	M	126.3	2.3	0.2	-1.2	-1.3
Plug3_N1_8	0	M	130.6	2.8	0.0	-0.7	-0.9
Plug3_N1_9	1	M	77.6	1.2	0.0	0.4	0.2
Plug3_N1_10	2	M	49.2	0.9	0.3	-0.1	-0.3
Plug3_N1_11	2	O	60.6	1.2	-0.1	-2.8	-0.7
Plug3_N1_12	1	O	99.2	1.7	0.1	-0.8	-0.9

Plug3_N1_13	0	O	125.1	2.9	0.4	0.1	0.0
Plug3_N1_14	-1	O	109.7	1.8	0.2	-0.1	-0.3
Plug3_N1_15	-2	O	119.9	2.0	0.1	-0.7	-0.9
Plug3_P1_1	5	O	224.5	2.2	-0.3	1.5	-0.2
Plug3_P1_2	4	O	199.6	2.4	-0.1	2.4	-0.4
Plug3_P1_3	3	O	171.0	2.7	-1.2	0.8	-0.2
Plug3_P1_4	1	O	144.4	3.0	0.1	1.3	0.1
Plug3_P1_5	0	O	121.6	2.1	0.1	-0.3	0.2
Plug3_P1_6	-1	O	150.4	1.9	0.6	-0.7	-0.9
Plug3_P1_7	-1	I	139.2	1.4	0.5	-1.3	0.3
Plug3_P1_8	0	I	122.4	2.8	-0.2	0.4	-0.2
Plug3_P1_9	1	I	122.5	2.1	-1.0	0.4	0.3
Plug3_P1_10	2	C	280.0	2.4	-0.4	-1.5	-0.3
Plug3_P1_11	3	C	175.6	2.8	-0.6	1.0	-0.3
Plug3_P1_12	4	C	200.2	2.2	0.2	1.8	0.0
Plug3_O2_1	3	I	101.1	2.0	0.5	0.6	0.0
Plug3_O2_2	2	I	117.5	2.8	0.1	-0.4	-0.1
Plug3_O2_3	1	I	128.2	1.9	0.3	-0.6	-0.1
Plug3_O2_4	0	I	133.2	2.1	0.2	-1.2	-1.2
Plug3_O2_5	-1	I	129.0	2.9	0.6	-0.7	-0.9
Plug3_O2_6	-1	C	136.7	2.7	0.3	-1.4	-0.2
Plug3_O2_7	0	C	138.2	1.6	0.3	-0.7	-0.4
Plug3_O2_8	1	C	136.2	1.7	0.6	-0.8	-0.2
Plug3_O2_9	2	C	120.8	2.7	0.5	0.3	0.1
Plug3_O2_10	3	C	68.7	1.5	-0.6	0.1	-0.1
Plug3_O2_11	3	O	115.7	1.7	0.2	0.6	-0.1
Plug3_O2_12	2	O	122.4	2.4	-0.3	0.8	0.4
Plug3_O2_13	1	O	134.1	2.3	0.4	-0.7	-0.6
Plug3_O2_14	0	O	144.0	2.2	0.5	-1.9	-0.4
Plug3_O2_15	-1	O	144.5	2.0	0.2	-2.0	-1.8
Plug3_Q1_1	5	I	179.7	2.6	0.1	2.6	-0.2
Plug3_Q1_2	4	C	140.5	1.9	0.3	0.3	0.0
Plug3_Q1_3	3	I	151.8	1.5	0.8	0.4	0.0
Plug3_Q1_4	2	C	141.1	2.3	0.3	-0.2	-0.3
Plug3_Q1_5	1	C	141.0	2.4	0.1	0.3	-0.8
Plug3_Q1_6	0	C	138.8	1.9	0.2	-1.2	-0.7
Plug3_Q1_7	3	C	152.0	2.0	0.1	0.6	0.1
Plug3_R1_1	4	I	157.4	2.6	-0.4	1.4	-0.1
Plug3_R1_2	3	I	137.0	2.4	-0.2	-0.1	0.1

Plug3_R1_3	2	I	136.8	1.5	0.4	-0.1	0.2
Plug3_R1_4	1	I	140.1	2.5	0.7	0.1	0.0
Plug3_R1_5	0	I	127.8	2.6	-0.1	-0.1	-0.7
Plug3_R1_6	0	C	125.4	2.9	-0.1	-1.4	-0.4
Plug3_R1_7	1	C	133.6	1.9	0.5	-0.1	-0.2
Plug3_R1_8	2	C	136.0	2.4	-0.3	0.2	-0.1
Plug3_R1_9	3	C	137.8	2.4	0.8	0.9	0.2
Plug3_R1_10	4	C	153.5	2.6	-1.3	0.8	0.1
Plug3_R1_11	4	O	159.2	2.8	0.2	-0.1	0.4
Plug3_R1_12	3	O	138.6	2.3	0.3	0.1	0.3
Plug3_R1_13	2	O	141.1	2.4	0.4	0.4	-0.1
Plug3_R1_14	1	O	132.5	2.9	-0.2	-1.2	-1.3
Plug3_R1_15	0	O	123.0	2.5	0.1	-1.4	-1.2
Plug3_R1_16	-1	C	128.2	2.9	0.0	-0.4	-0.4
Plug3_R1_17	-2	C	133.7	2.3	-0.1	-0.5	-1.1
Plug3_R1_18	-3	C	126.1	2.5	0.0	-1.7	-1.7
Plug3_R1_19	-4	C	130.6	2.7	0.2	-0.1	-0.7
Plug3_R1_20	-5	C	127.3	2.7	0.1	-1.1	-1.8
Plug3_R1_21	-6	C	127.2	2.5	0.2	-2.3	-1.2
Plug3_R1_22	-7	C	124.2	2.6	0.1	-2.0	-1.2
Plug3_R1_23	-8	C	126.5	2.5	-0.1	-2.1	-1.5
Plug3_R1_24	-9	C	135.7	2.1	-0.3	-1.6	-1.1
Plug3_R1_25	-10	C	119.0	1.9	0.0	0.0	0.5
Plug3_R1_26	-11	C	128.7	2.3	-0.1	-1.9	-1.3
Plug3_R1_27	-12	C	123.6	2.7	-0.3	-0.5	-0.9
Plug3_R1_28	-13	C	124.5	2.7	0.4	-2.0	-1.1
Plug3_R1_29	-14	C	142.9	1.6	0.1	-2.1	-1.9
Plug3_R1_30	-15	C	139.6	1.6	0.0	-1.3	-0.3
Plug3_R1_31	-16	C	143.1	2.3	0.4	-2.3	-1.3
Plug3_R1_32	-17	C	130.4	2.0	0.2	0.1	0.0
Plug3_R1_33	-18	C	134.6	2.4	-0.1	0.4	-0.1
Plug3_R1_34	-19	C	138.3	2.4	0.1	-0.2	0.4
Plug3_R1_35	-20	C	152.3	2.2	-0.1	-2.4	-0.2
Plug3_S1_1	2	I	97.2	2.4	-0.2	0.0	-0.2
Plug3_S1_2	1	I	111.9	2.5	0.4	-0.1	-0.1
Plug3_S1_3	0	I	135.2	2.8	0.0	0.7	-0.3
Plug3_S1_4	-1	I	112.6	3.2	0.2	-0.2	-0.5
Plug3_S1_5	-2	I	48.5	4.9	1.0	-0.4	-0.2
Plug3_S1_6	-1	C	118.8	2.5	0.2	-0.2	-0.3

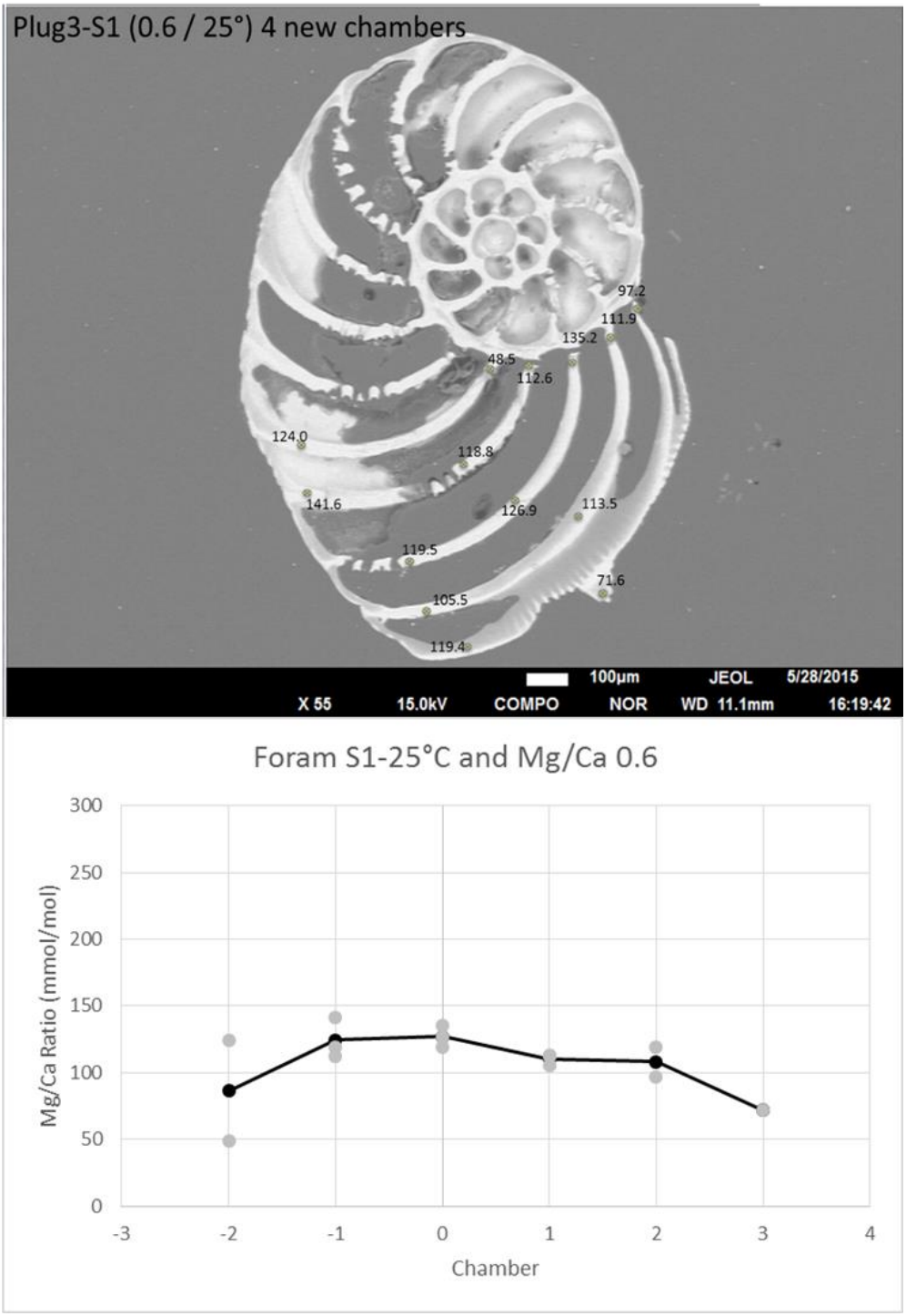
Plug3_S1_7	0	C	126.9	2.6	0.1	0.4	0.3
Plug3_S1_8	1	C	113.5	2.6	0.0	0.1	0.0
Plug3_S1_9	3	C	71.6	1.1	-0.6	0.6	0.0
Plug3_S1_10	3	O	119.4	2.8	1.0	0.5	-0.2
Plug3_S1_11	1	O	105.5	2.5	0.2	0.0	-0.5
Plug3_S1_12	0	O	119.5	2.4	0.5	-3.8	-0.8
Plug3_S1_13	-1	O	141.6	2.2	0.2	0.6	0.6
Plug3_S1_14	-2	O	124.0	2.7	0.0	0.0	-0.2
Plug4_T1_1	1	I	86.4	1.4	-0.6	0.6	-0.1
Plug4_T1_2	0	I	119.3	2.6	0.9	0.0	0.1
Plug4_T1_3	-1	I	123.1	2.2	0.4	-1.8	-0.4
Plug4_T1_4	-2	I	124.1	2.6	-0.1	-0.6	-0.6
Plug4_T1_5	-3	O	116.1	1.5	0.1	-0.6	-0.1
Plug4_T1_6	-2	O	112.2	1.8	0.7	0.2	-0.1
Plug4_T1_7	-1	O	113.3	2.3	0.8	-0.4	-0.1
Plug4_T1_8	-1	C	124.0	2.2	0.8	-0.9	0.1
Plug4_T1_9	-2	C	118.4	2.3	0.7	-0.4	-0.1
Plug4_T1_10	-4	I	117.2	3.0	0.2	-0.6	-0.9
Plug4_T1_11	-4	O	115.9	2.8	0.4	-0.5	-0.4
Plug4_W1_1	2	O	153.2	2.4	0.3	2.2	0.7
Plug4_W1_2	1	O	131.6	3.7	0.6	-0.1	-0.2
Plug4_W1_3	0	O	118.5	2.7	-0.2	-0.5	0.8
Plug4_W1_4	-1	O	126.1	2.5	0.0	0.5	0.1
Plug4_W1_5	-2	O	117.6	1.7	0.4	-0.3	-0.2
Plug4_W1_6	-2	C	134.1	2.6	-0.1	-2.6	-0.6
Plug4_W1_7	-1	C	132.7	3.2	-0.4	-0.7	0.0
Plug4_W1_8	0	C	132.2	3.1	-0.1	-0.1	0.3
Plug4_W1_9	1	C	141.9	1.7	0.5	0.7	0.3
Plug4_W1_10	2	C	154.6	1.6	-1.2	2.2	0.6
Plug4_Y13_1	3	I	145.0	1.9	0.5	0.1	-0.1
Plug4_Y13_2	2	I	130.5	2.4	0.5	0.2	0.3
Plug4_Y13_3	1	I	134.1	2.6	0.2	-1.8	-0.8
Plug4_Y13_4	0	I	145.8	2.1	0.2	0.0	-0.3
Plug4_Y13_5	-1	I	131.9	2.4	0.4	0.4	-0.1
Plug4_Y13_6	-1	C	124.3	2.9	0.3	0.2	0.2
Plug4_Y13_7	0	C	123.4	1.9	-0.1	0.1	-0.2
Plug4_Y13_8	1	C	139.0	2.2	-0.3	0.2	0.2
Plug4_Y13_9	2	C	136.5	2.0	0.5	0.2	-0.3
Plug4_Y13_10	3	C	135.2	2.7	0.2	0.0	-0.2

Plug4_Y13_11	3	O	129.7	2.8	0.4	-0.1	-0.1
Plug4_Y13_12	2	O	134.6	2.3	0.2	0.6	0.0
Plug4_Y13_13	1	O	123.3	2.3	-0.1	0.4	-0.1
Plug4_Y13_14	0	O	135.2	2.6	0.4	0.3	0.2
Plug4_Y13_15	-1	O	133.8	2.2	0.1	-0.2	-0.1
Plug5_BB2_1	3	O	71.3	1.3	0.1	0.4	0.1
Plug5_BB2_2	2	O	97.6	2.8	0.2	0.4	-0.1
Plug5_BB2_3	1	O	93.7	1.9	0.0	0.9	-0.5
Plug5_BB2_4	0	O	134.7	2.4	0.6	-1.8	0.2
Plug5_BB2_5	-1	O	135.2	2.1	0.4	-2.1	-0.9
Plug5_BB2_6	-1	C	120.9	2.5	0.3	-0.1	0.0
Plug5_BB2_7	0	C	108.7	2.6	0.4	0.5	0.3
Plug5_BB2_8	1	C	97.3	2.3	0.9	-0.5	0.2
Plug5_BB2_9	2	C	111.8	1.8	0.2	0.9	-0.3
Plug5_BB2_10	3	C	56.7	1.6	-0.9	0.8	0.1
Plug5_BB2_11	3	I	64.9	1.1	-0.9	1.2	-0.4
Plug5_BB2_12	2	I	97.4	2.2	0.3	0.5	0.0
Plug5_BB2_13	1	I	139.3	2.3	0.8	0.3	0.1
Plug5_BB2_14	0	I	123.6	2.1	0.5	-1.4	-0.3
Plug5_BB2_15	-1	I	108.3	2.5	0.5	-1.1	-0.4
Plug5_BB2_16	-1	O	121.8	2.7	0.4	-1.7	-0.1
Plug5_BB2_17	-3	O	107.2	2.2	0.2	0.0	0.1
Plug5_BB2_18	-3	O	152.8	2.7	-1.9	-1.2	0.4
Plug5_BB2_19	-5	O	100.9	2.6	0.6	0.5	-0.1
Plug5_BB2_20	-7	O	122.9	2.0	0.3	-0.1	0.1
Plug5_BB2_21	-9	O	138.2	1.9	0.4	0.2	0.4
Plug5_X1_1	3	I	125.6	3.0	0.3	0.1	0.0
Plug5_X1_2	2	I	127.8	2.3	0.4	-0.3	0.0
Plug5_X1_3	1	I	121.4	3.2	0.4	0.0	-0.5
Plug5_X1_4	0	I	116.2	3.0	-0.1	-0.2	-0.5
Plug5_X1_5	-1	I	128.2	2.2	-0.4	-0.1	0.1
Plug5_X1_6	-1	C	139.9	2.3	-0.8	0.2	0.4
Plug5_X1_7	0	C	122.7	2.0	0.2	-0.2	0.0
Plug5_X1_8	1	C	132.3	2.2	0.7	-2.6	-1.3
Plug5_X1_9	2	C	156.3	2.5	-1.0	0.6	0.3
Plug5_X1_10	3	C	136.8	3.4	0.4	0.5	0.7
Plug5_X1_11	3	O	136.1	2.2	0.4	0.0	0.6
Plug5_X1_12	2	O	144.0	2.5	0.0	0.1	0.1
Plug5_X1_13	1	O	145.0	1.8	0.3	0.1	-0.5

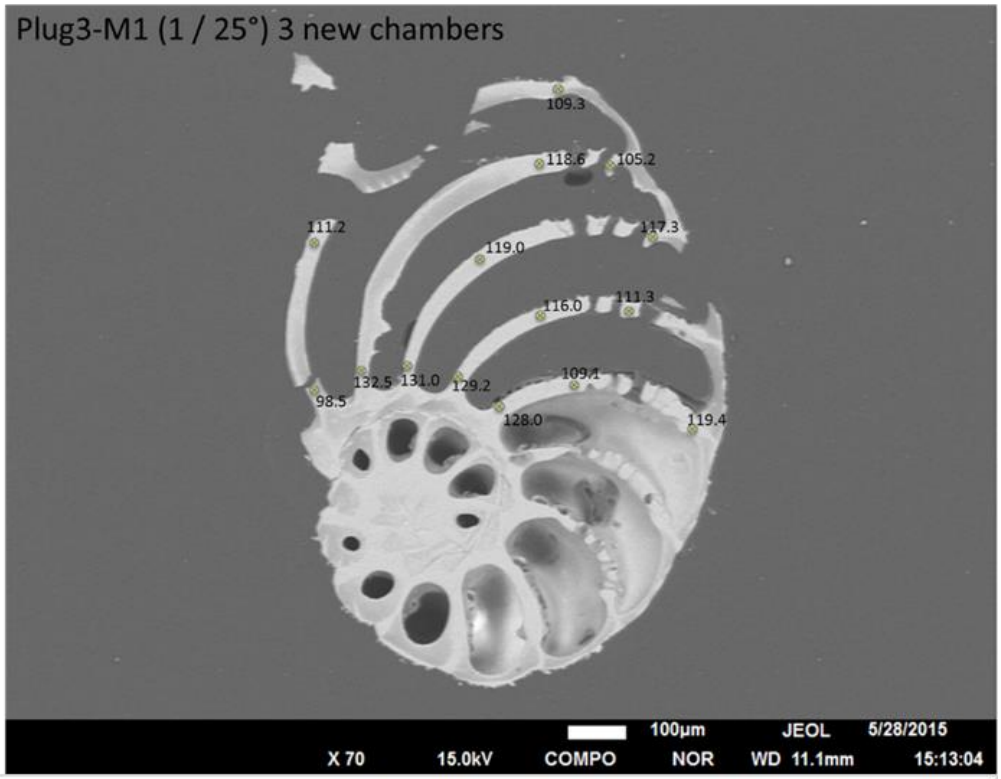
Plug5_X1_14	0	O	143.5	2.2	0.0	-0.3	0.5
Plug5_X1_15	-1	O	136.7	2.3	0.0	-2.6	-0.6
Plug5_X1_16	-1	O	126.8	2.7	0.3	-0.9	-0.2
Plug5_Z1_1	3	I	156.7	3.4	0.0	0.1	-0.3
Plug5_Z1_2	2	I	139.9	1.9	0.8	0.6	-0.2
Plug5_Z1_3	1	I	147.5	2.5	0.3	0.1	0.1
Plug5_Z1_4	0	I	142.2	2.7	-0.2	0.4	-0.3
Plug5_Z1_5	-1	I	115.3	3.1	-0.3	0.4	0.1
Plug5_Z1_6	-1	C	118.7	2.4	0.7	-0.1	-0.2
Plug5_Z1_7	0	C	129.7	3.0	0.1	0.1	0.3
Plug5_Z1_8	1	C	123.0	2.7	0.1	-0.5	-0.1
Plug5_Z1_9	2	C	126.8	2.7	0.8	-0.6	0.2
Plug5_Z1_10	2	O	126.5	1.8	-0.3	0.1	-0.2
Plug5_Z1_11	1	O	137.5	2.1	0.3	0.6	-0.1
Plug5_Z1_12	0	O	128.2	2.5	-0.1	0.4	0.0
Plug5_Z1_13	-1	O	123.0	2.5	0.2	-1.4	-0.2
Plug5_Z1_14	3	C	156.0	2.9	-0.2	0.0	0.3
Plug5_Z1_15	3	I	152.3	3.1	0.3	1.2	-0.1

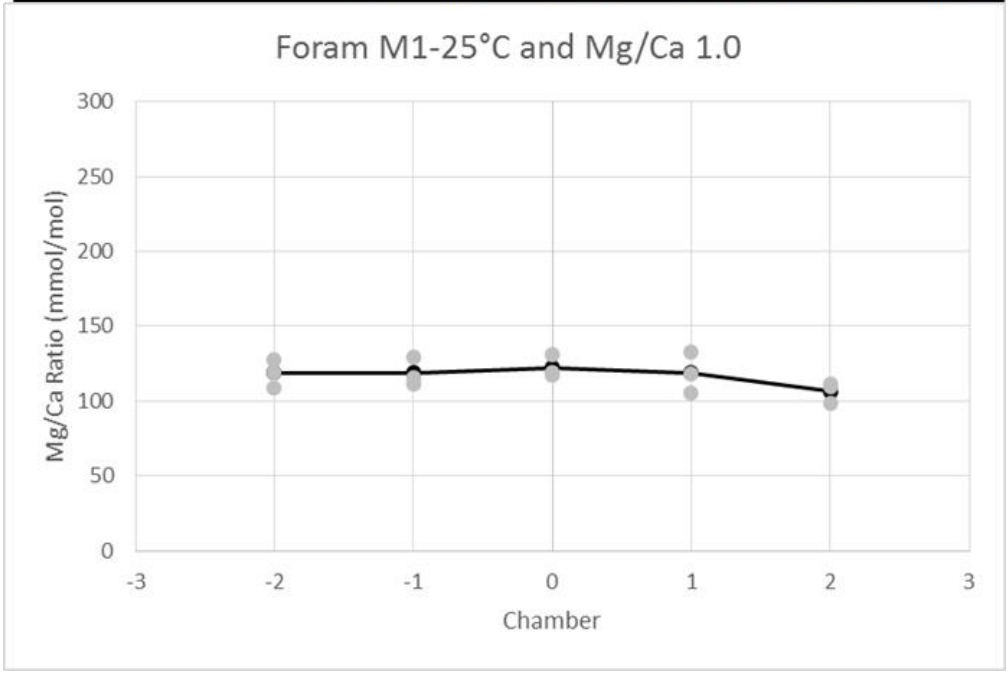
APPENDIX C. CULTURED FORAM GEOCHEMISTRY-SEM OVERLAYS

The following figures show SEM and geochemical data for each foraminifer analyzed by electron microprobe as part of the peneroplid study in Chapter III. Below the SEM image is a graph of the average Mg/Ca per chamber for this foraminifera. Gray dots indicate all values and black dots indicate chamber averages. Chamber 0 signifies the start of the experimental time period.

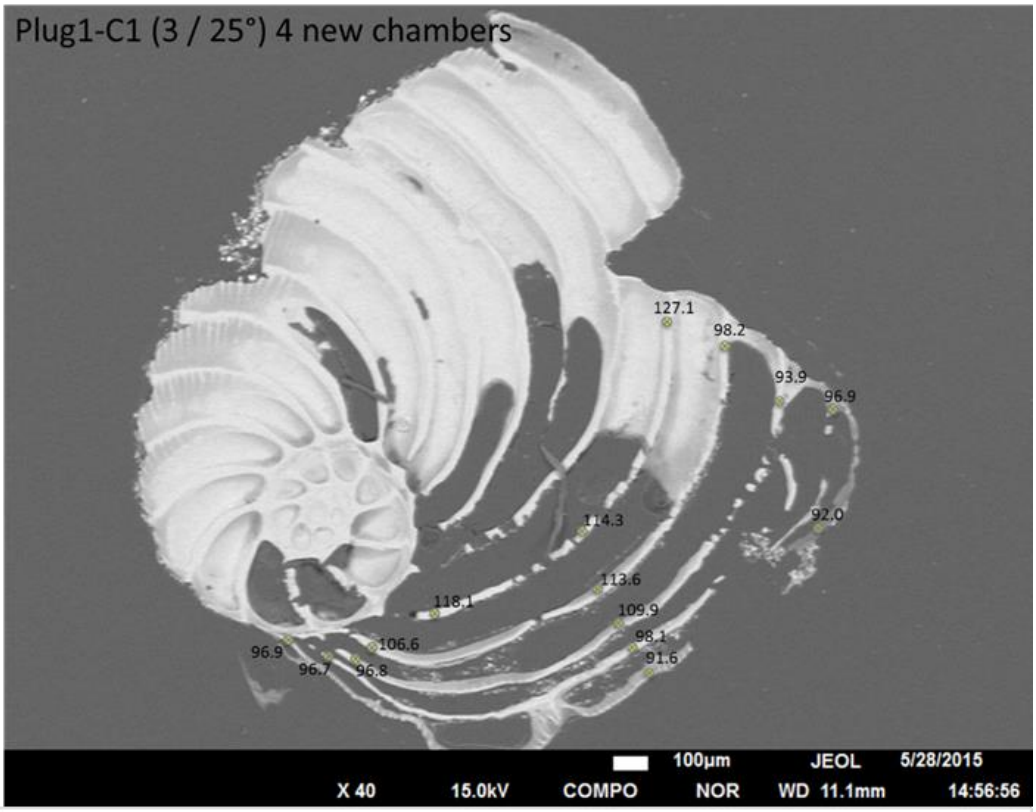


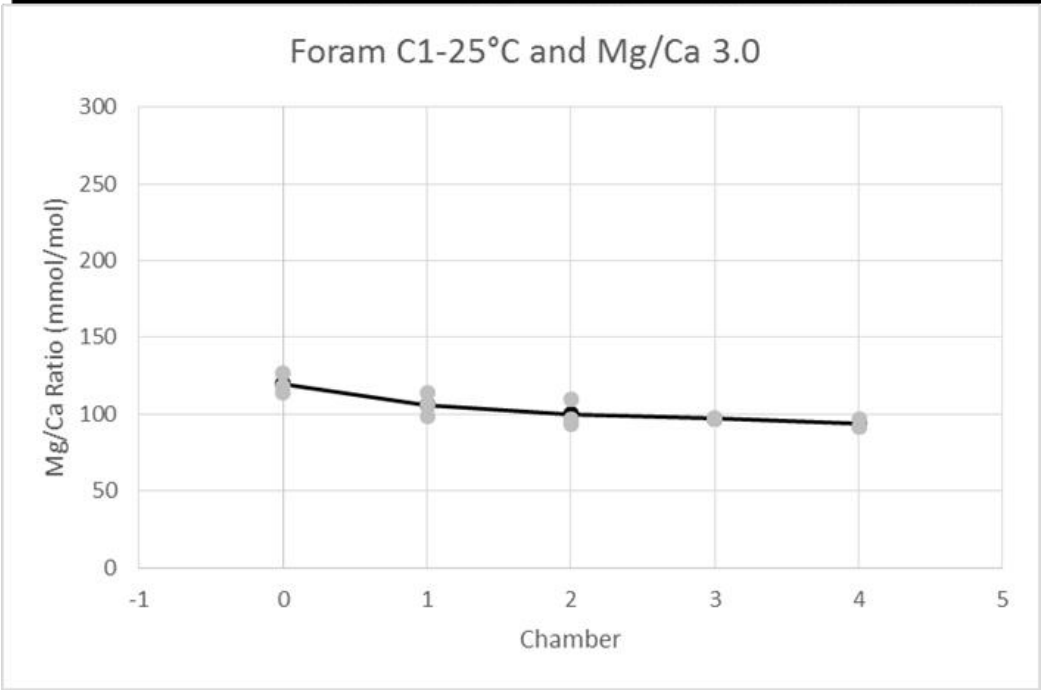
S1: SEM backscatter photomicrograph of foraminifera S1 that was placed in experimental seawater with a temperature of 25°C and a Mg/Ca ratio of 0.6. It grew 4 new chambers during the experimental process. One chamber (outermost chamber) was heavily damaged and nearly destroyed during sample processing.



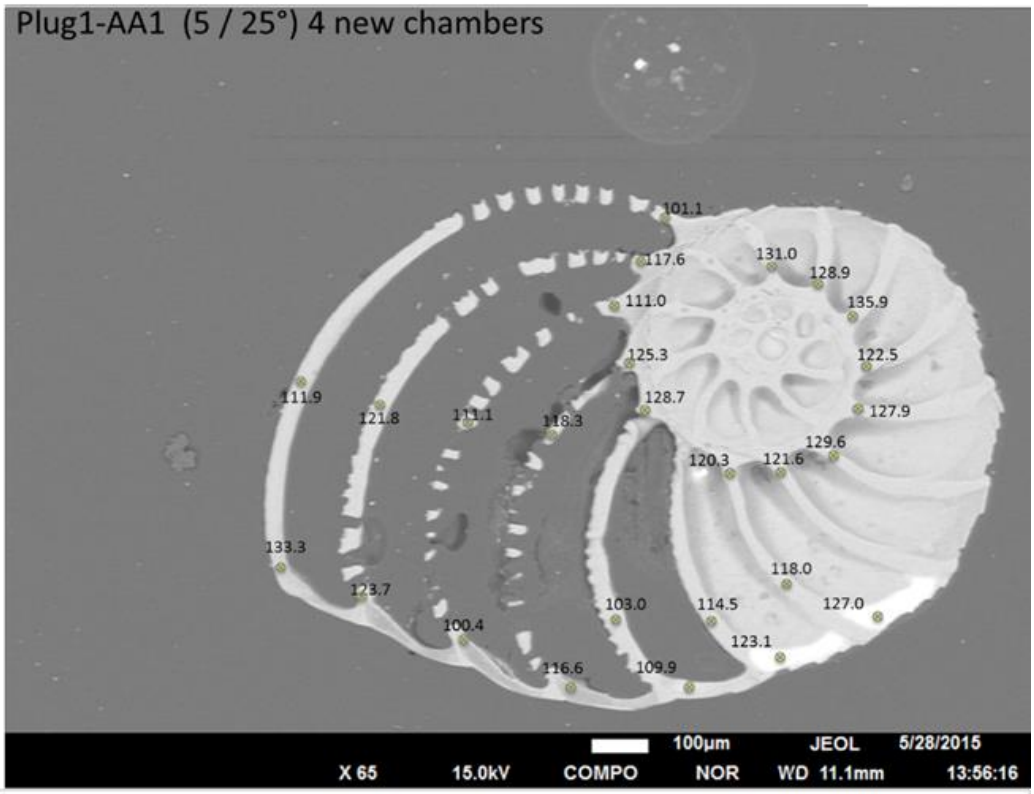


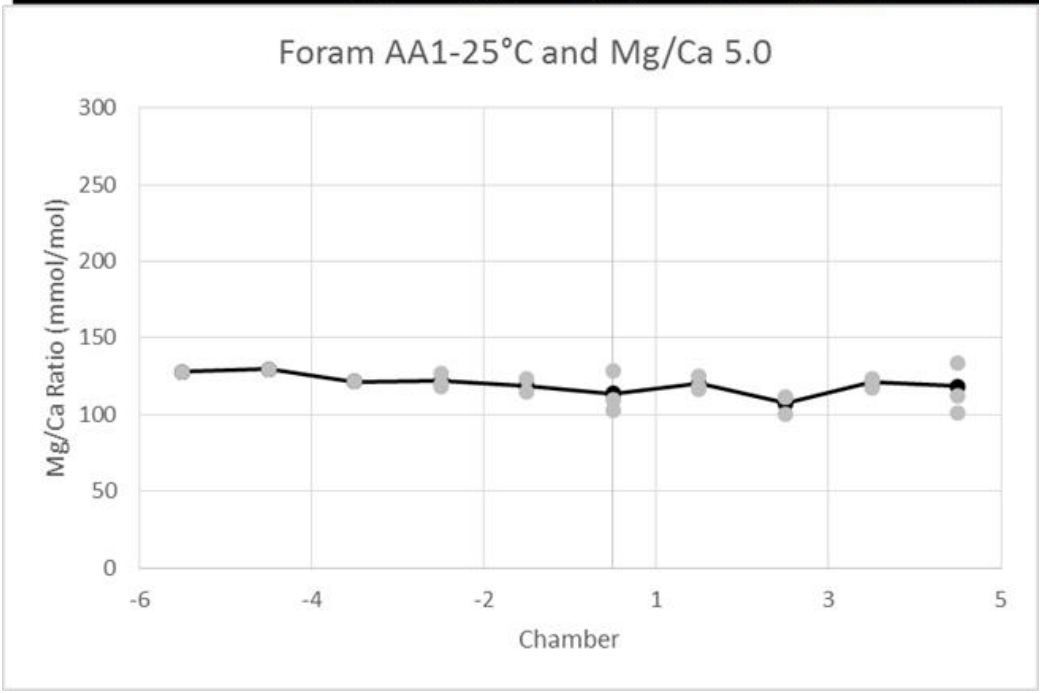
M1: SEM backscatter photomicrograph of foraminifera M1 that was placed in experimental seawater with a temperature of 3°C and a Mg/Ca ratio of 1.0. It grew 3 new chambers during the experimental process. One of those chambers (outermost one) was lost during sample processing.



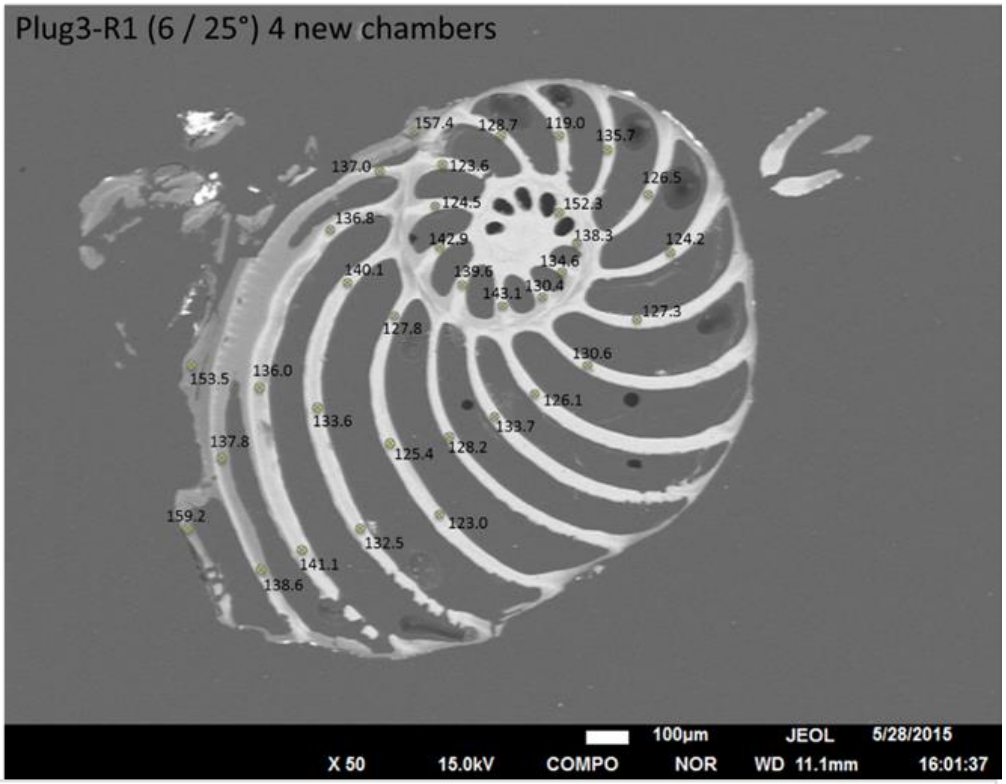


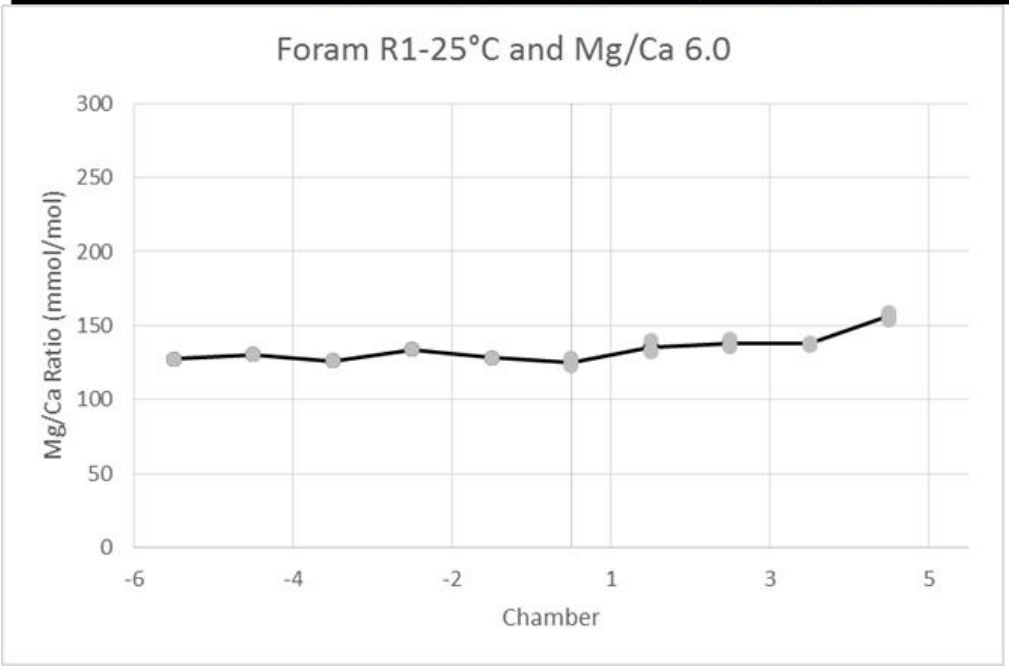
C1: SEM backscatter photomicrograph of foraminifera C1 that was placed in experimental seawater with a temperature of 25°C and a Mg/Ca ratio of 3.0. It grew 4 new chambers during the experimental process.



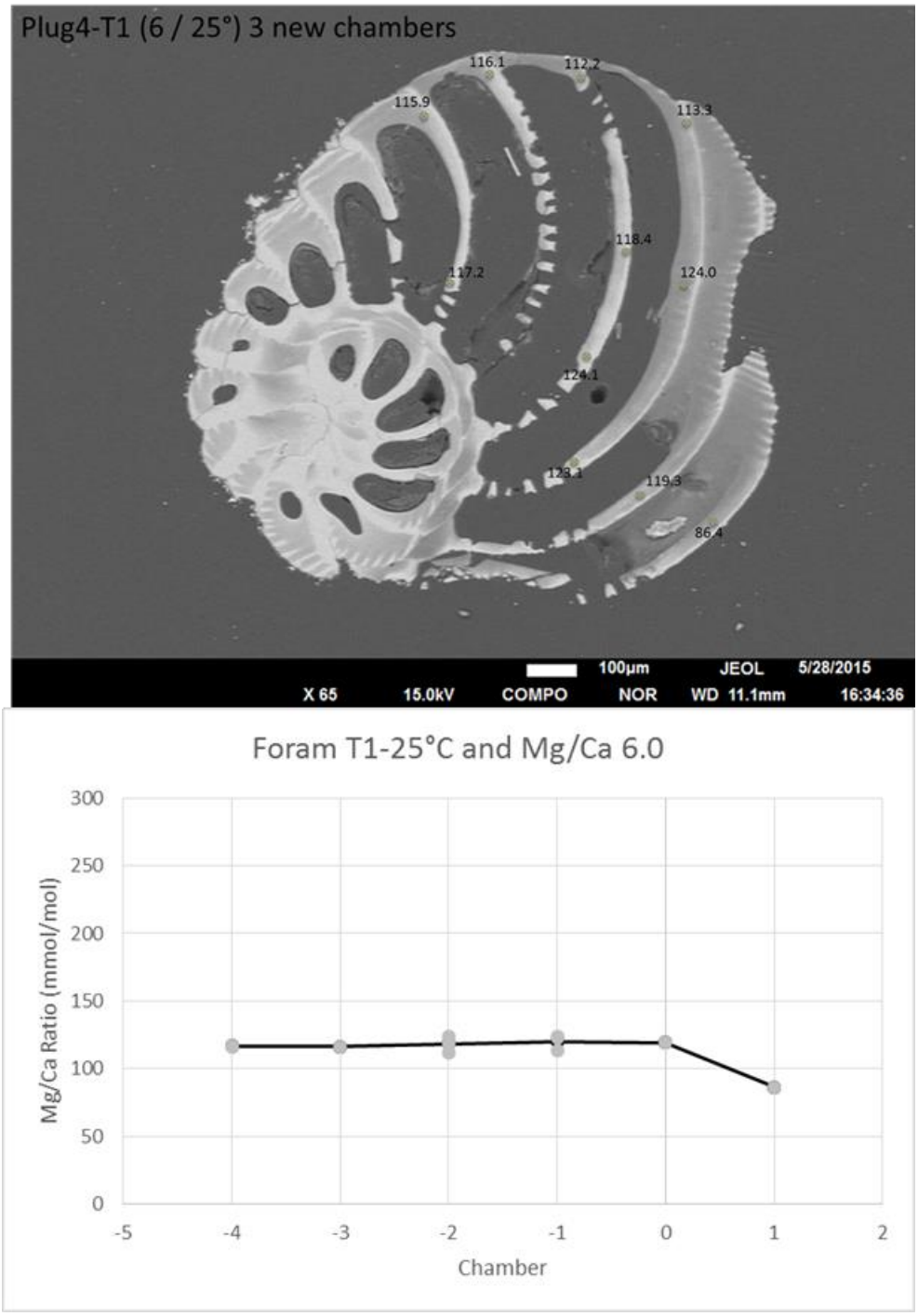


AA1: SEM backscatter photomicrograph of foraminifera AA1 that was placed in experimental seawater with a temperature of 25°C and a Mg/Ca ratio of 5.0. It grew 4 new chambers during the experimental process.

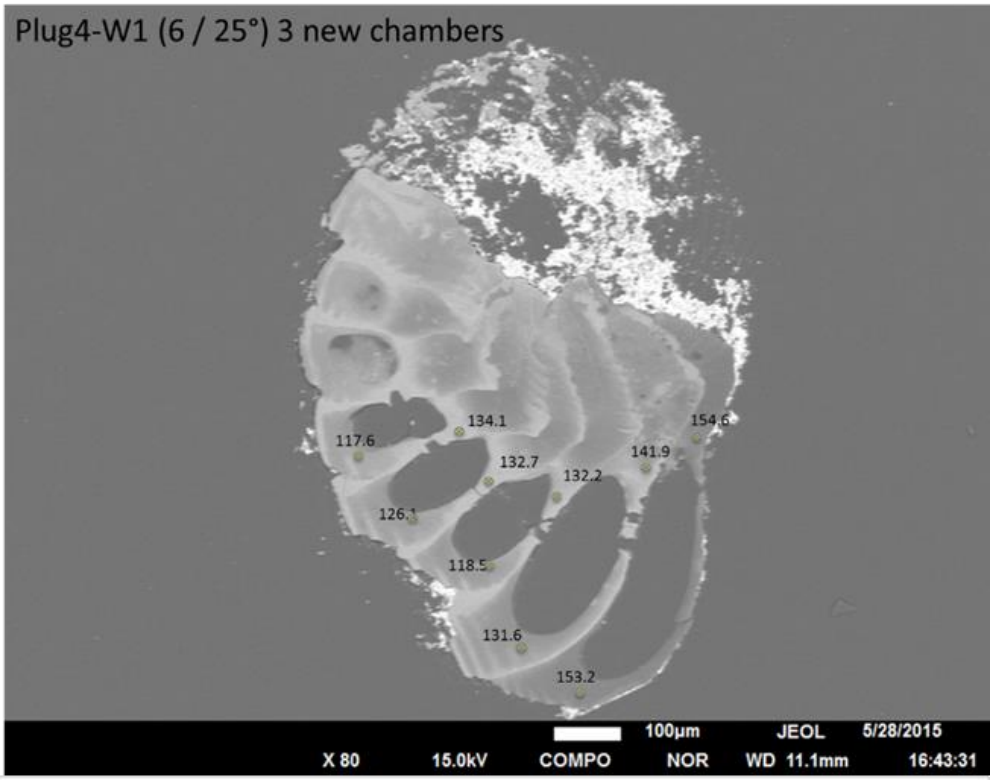


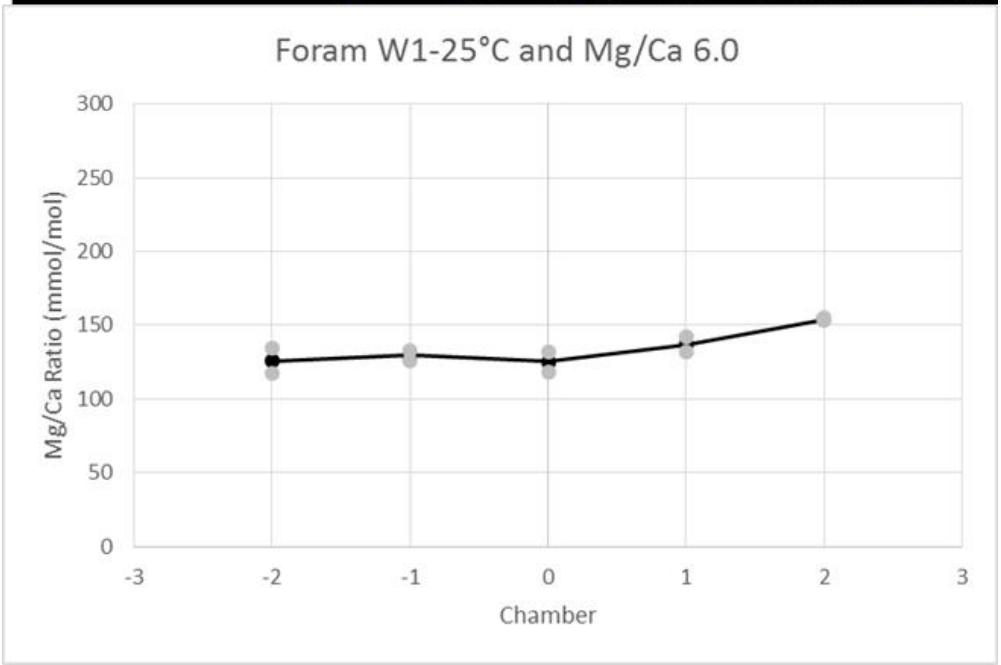


R1: SEM backscatter photomicrograph of foraminifera R1 that was placed in experimental seawater with a temperature of 25°C and a Mg/Ca ratio of 6.0. It grew 4 new chambers during the experimental process. One of those chambers (outermost one) was heavily damaged, as seen aboce, during sample processing.

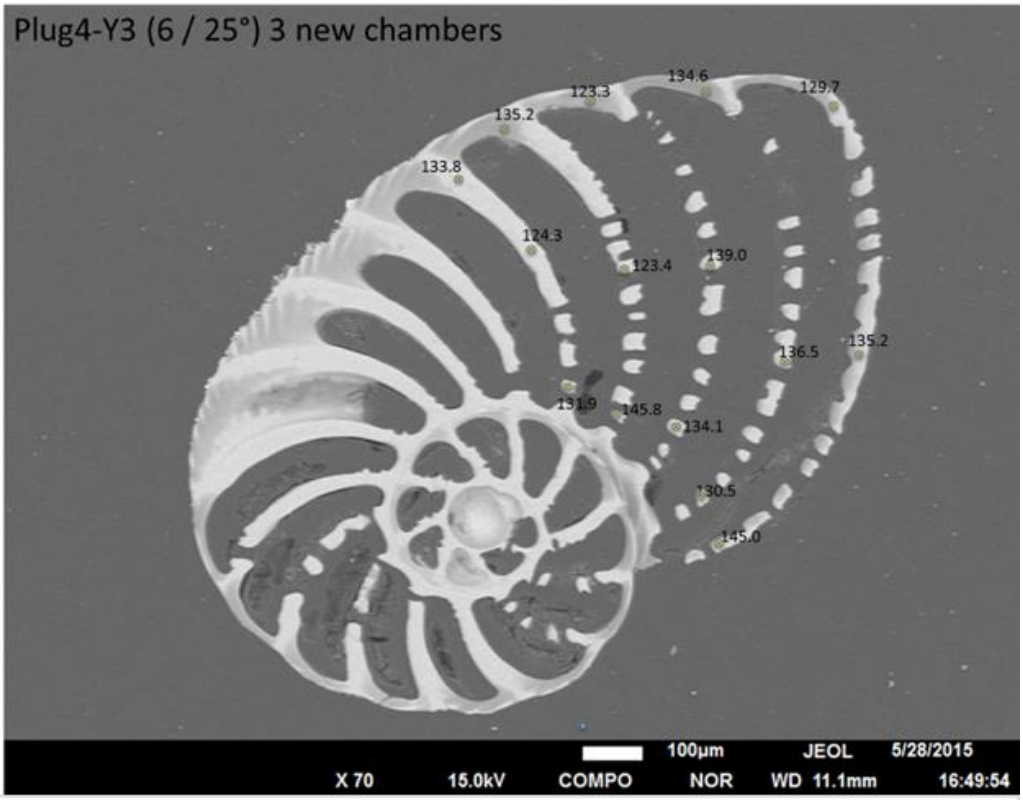


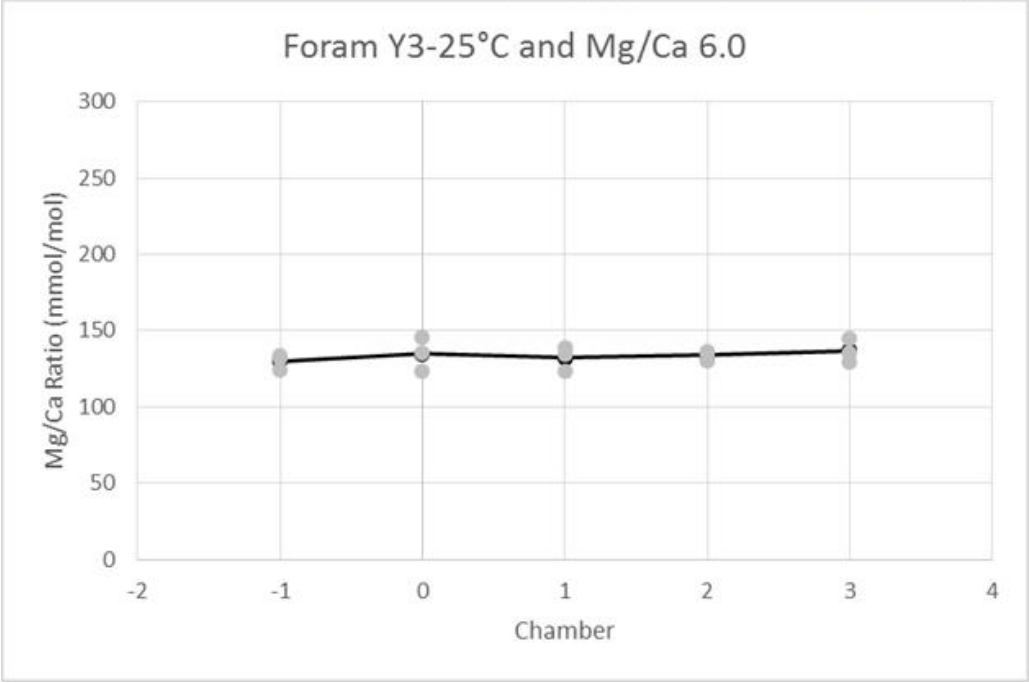
T1: SEM backscatter photomicrograph of foraminifera T1 that was placed in experimental seawater with a temperature of 25°C and a Mg/Ca ratio of 6.0. It grew 3 new chambers during the experimental process. Two of those chambers (outermost two) was lost during sample processing, and only a small fraction of a heavily damaged chamber of new growth remained.



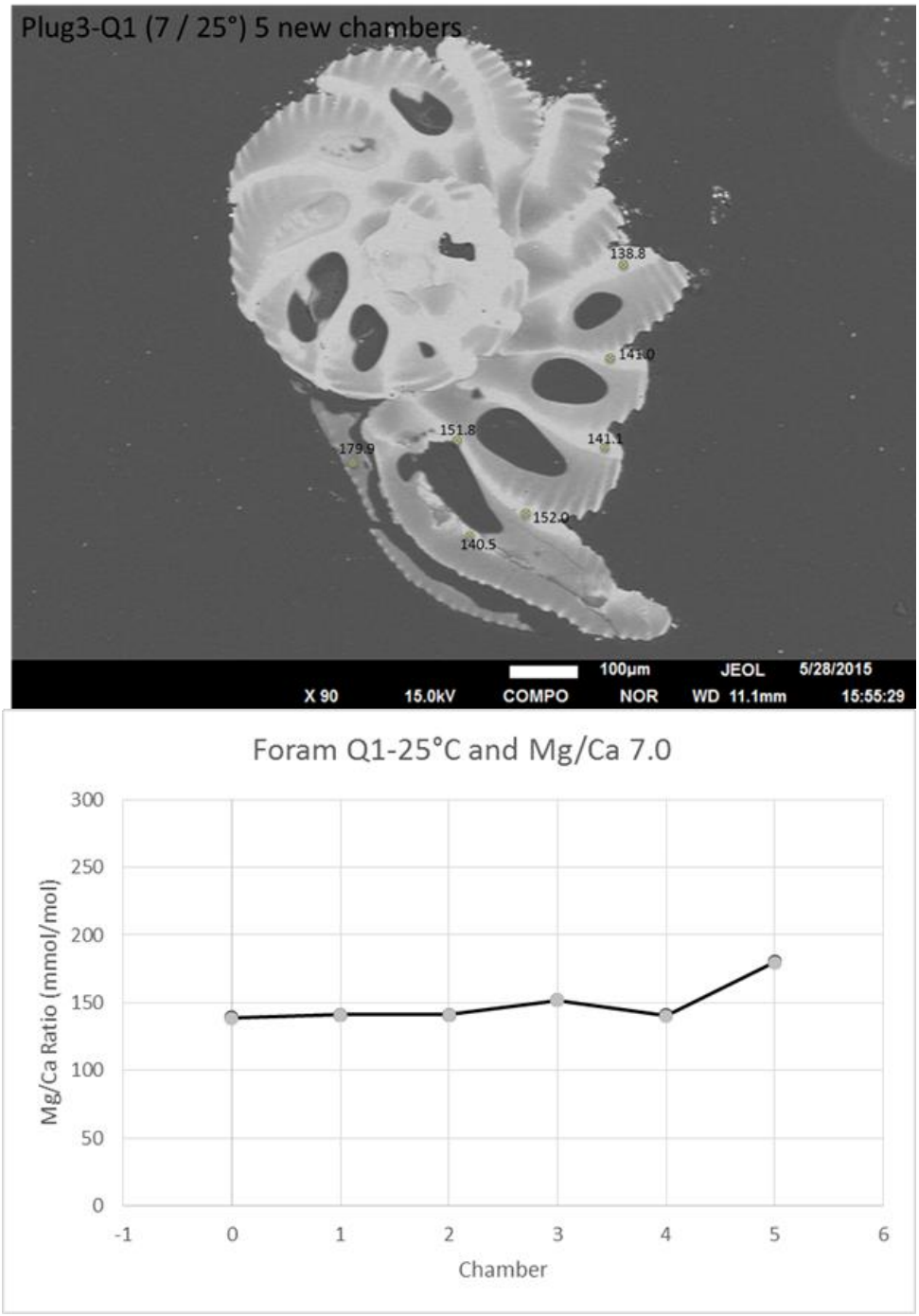


W1: SEM backscatter photomicrograph of foraminifera W1 that was placed in experimental seawater with a temperature of 25°C and a Mg/Ca ratio of 6.0. It grew 3 new chambers during the experimental process. One of those chambers (outermost one) was lost during sample processing.

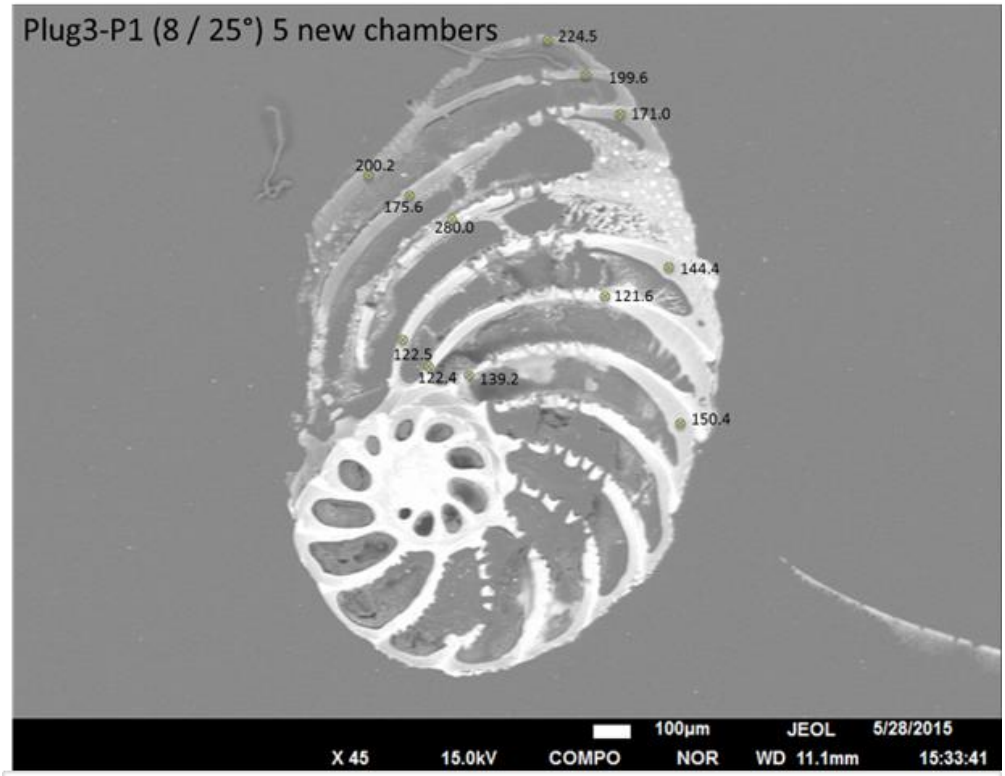


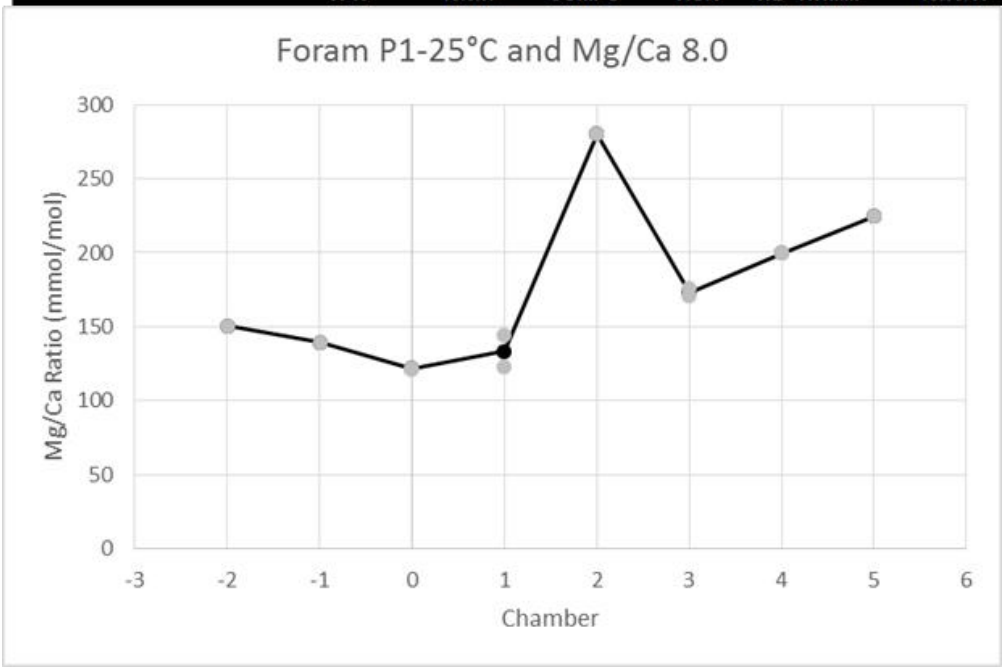


Y3: SEM backscatter photomicrograph of foraminifera Y3 that was placed in experimental seawater with a temperature of 25°C and a Mg/Ca ratio of 6.0. It grew 3 new chambers during the experimental process.

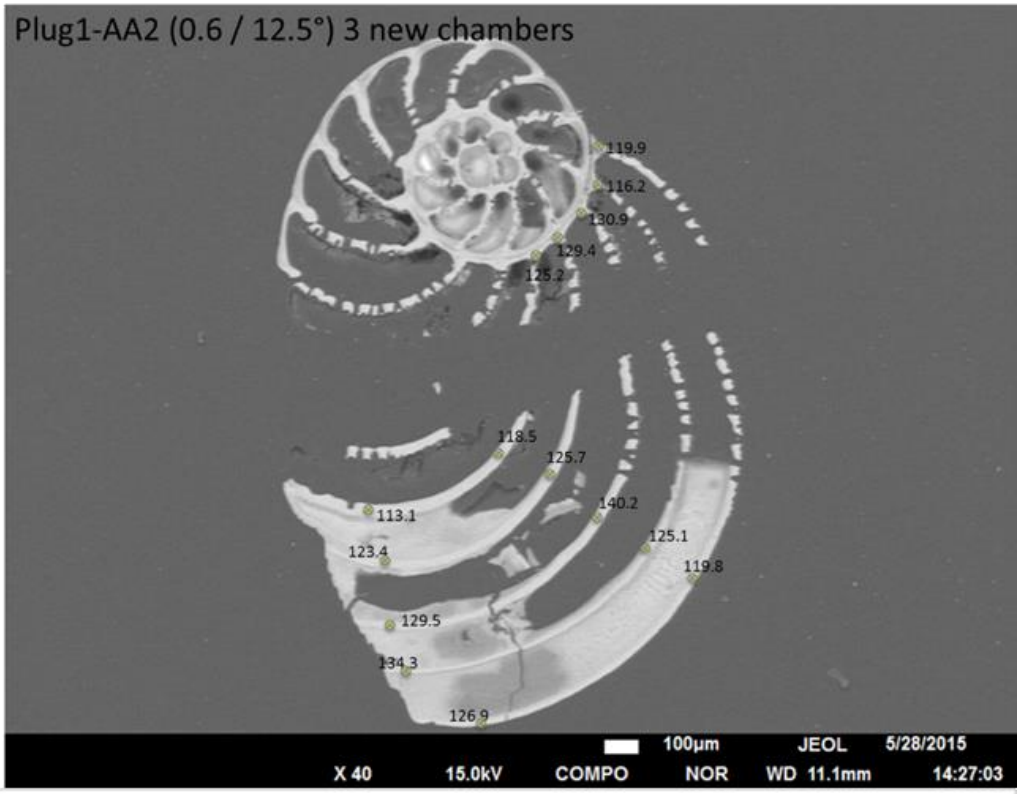


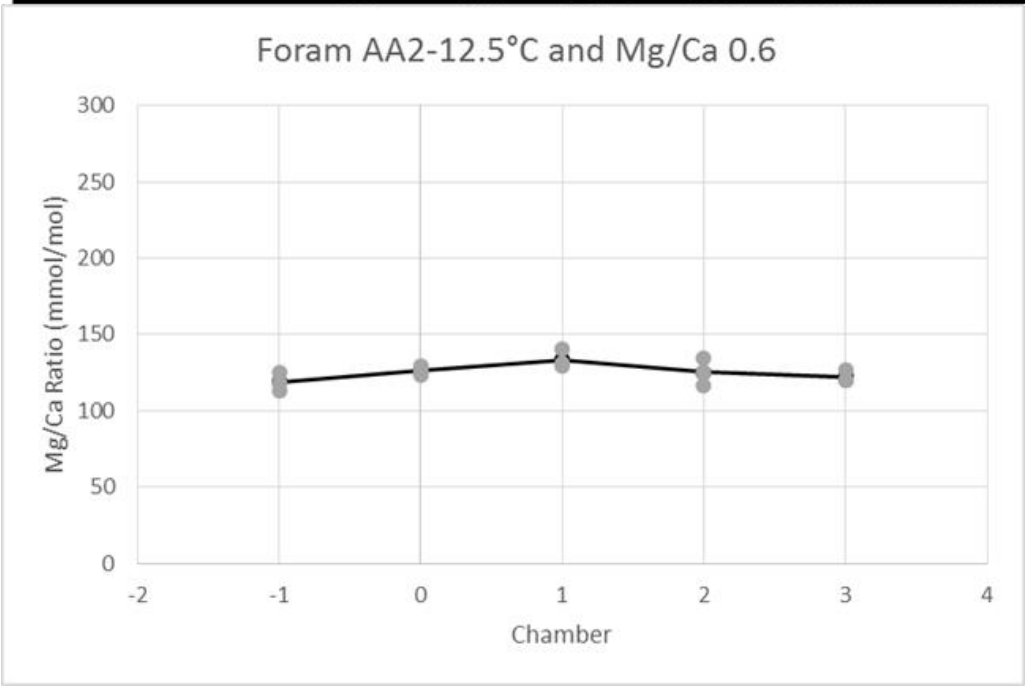
Q1: SEM backscatter photomicrograph of foraminifera Q1 that was placed in experimental seawater with a temperature of 25°C and a Mg/Ca ratio of 7.0. It grew 4 new chambers during the experimental process. One of those chambers (outermost one) was heavily damaged during sample preparation, as seen above.



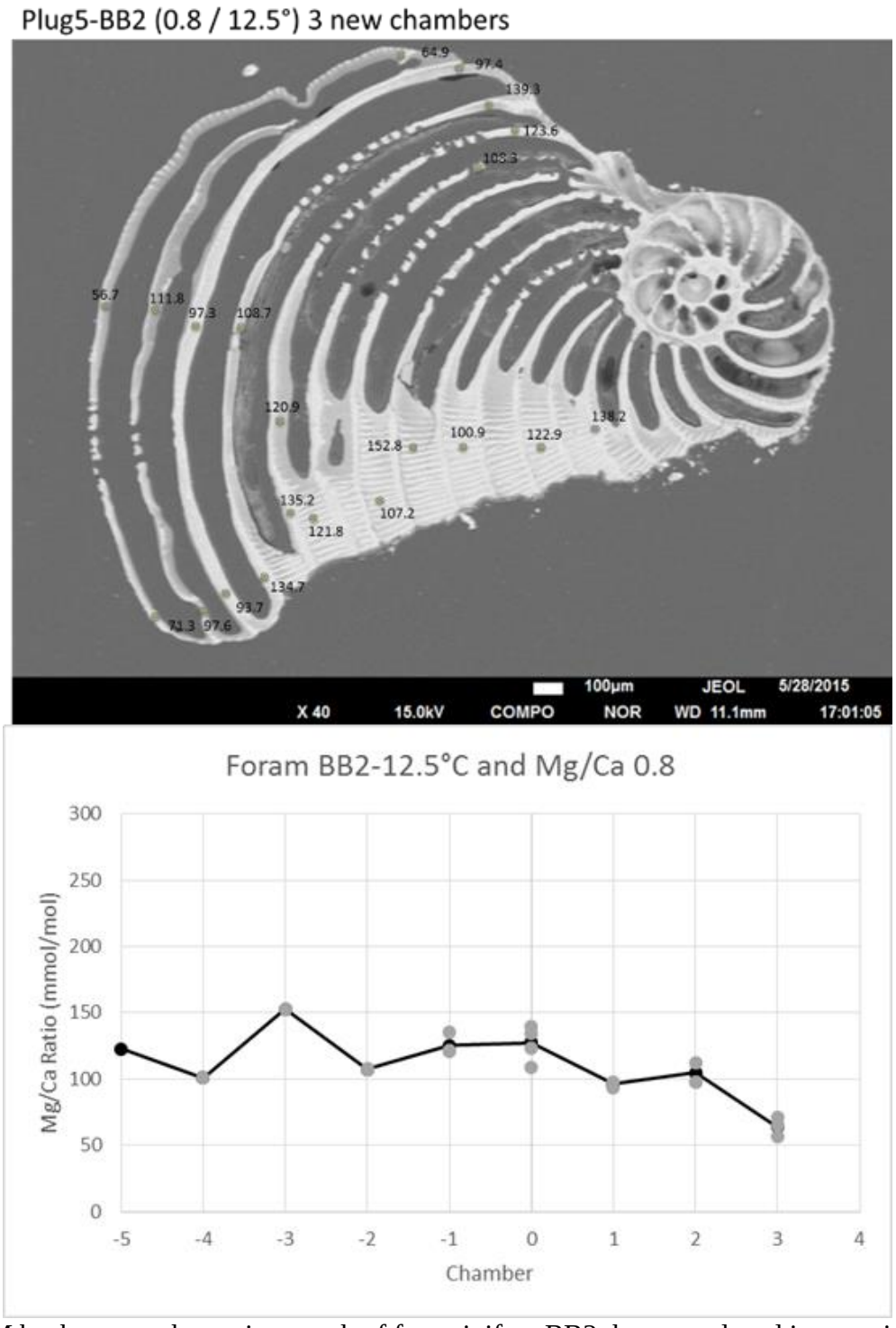


P1: SEM backscatter photomicrograph of foraminifera P1 that was placed in experimental seawater with a temperature of 25°C and a Mg/Ca ratio of 8.0. It grew 5 new chambers during the experimental process. One of those chambers (outermost one) was heavily damaged during sample processing.

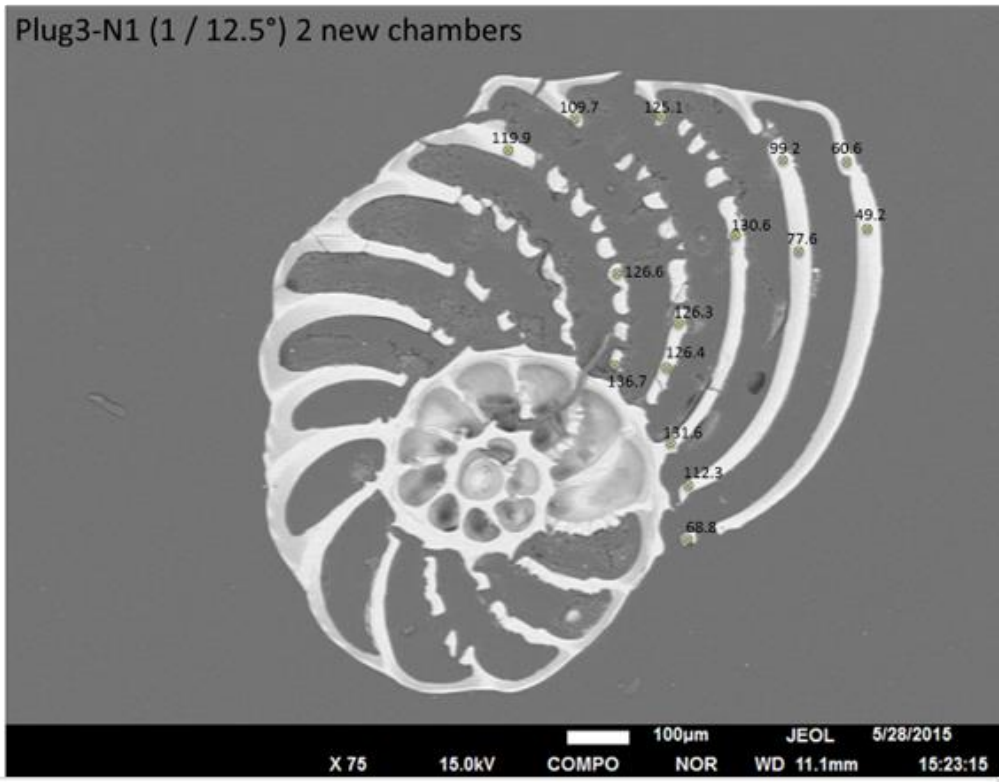


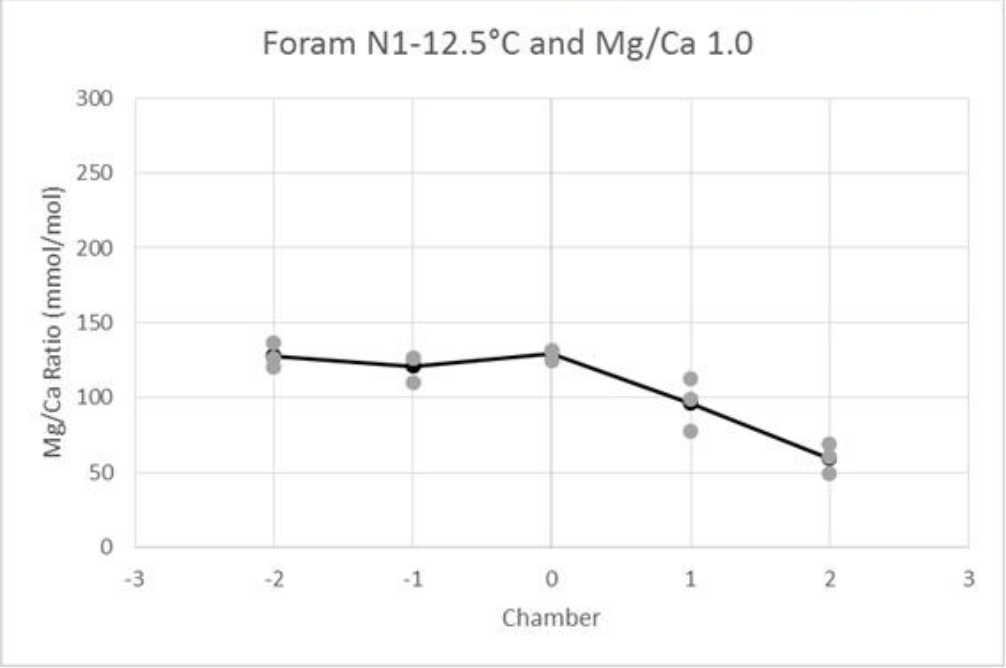


AA2: SEM backscatter photomicrograph of foraminifera AA2 that was placed in experimental seawater with a temperature of 12.5°C and a Mg/Ca ratio of 0.6. It grew 3 new chambers during the experimental process.

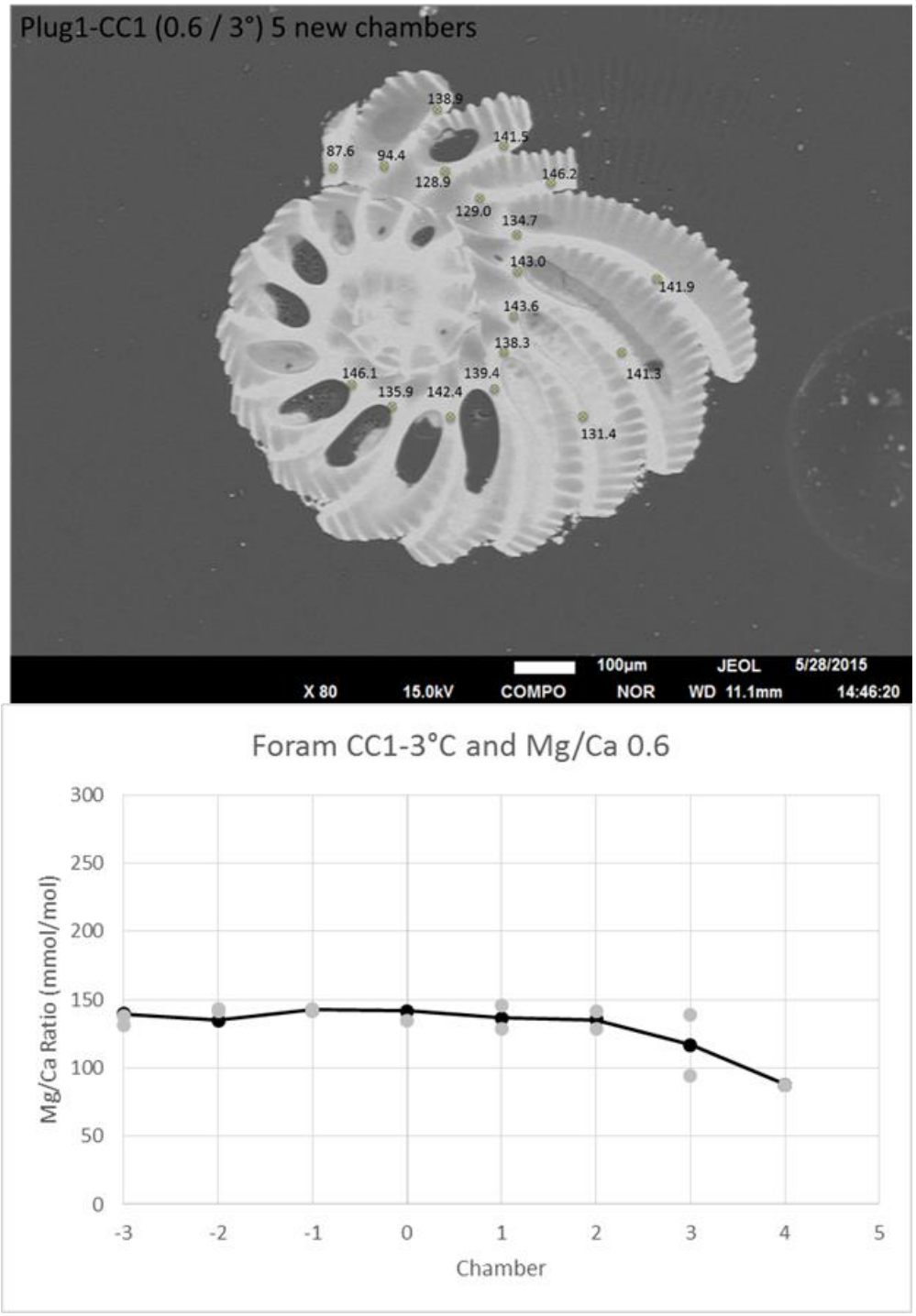


BB2: SEM backscatter photomicrograph of foraminifera BB2 that was placed in experimental seawater with a temperature of 12.5°C and a Mg/Ca ratio of 0.8. It grew 3 new chambers during the experimental process.

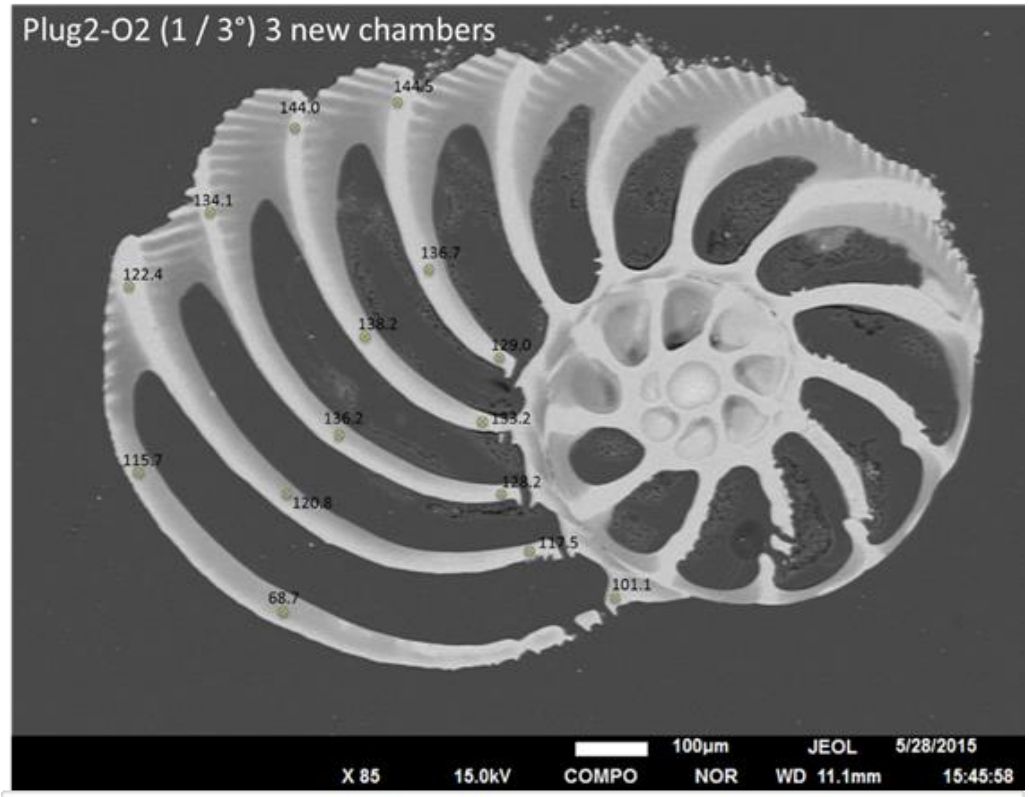


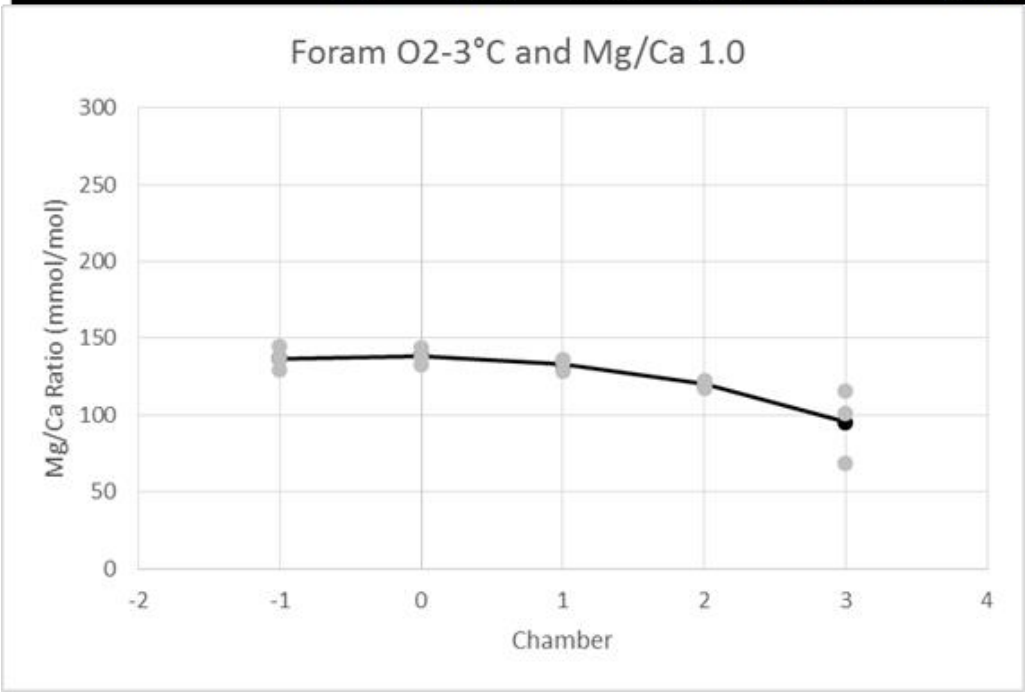


N1: SEM backscatter photomicrograph of foraminifera N1 that was placed in experimental seawater with a temperature of 12.5°C and a Mg/Ca ratio of 1.0. It grew 3 new chambers during the experimental process.

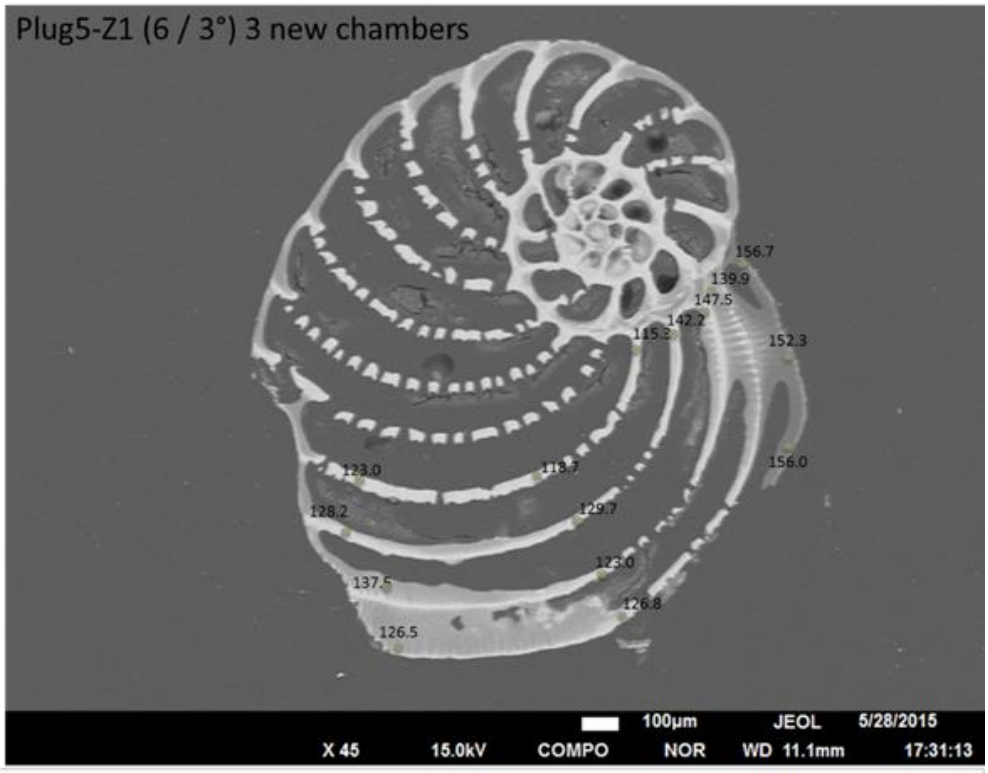


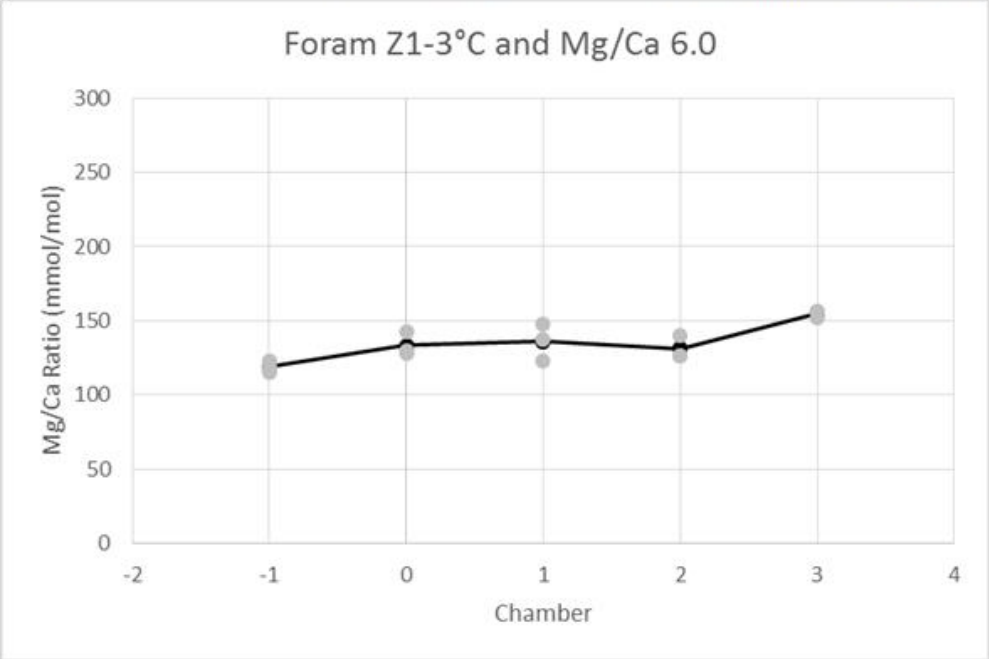
CC1: SEM backscatter photomicrograph of foraminifera CC1 that was placed in experimental seawater with a temperature of 3°C and a Mg/Ca ratio of 0.6. It grew 4 new chambers during the experimental process. One of those chambers (outermost one) was lost during sample processing.



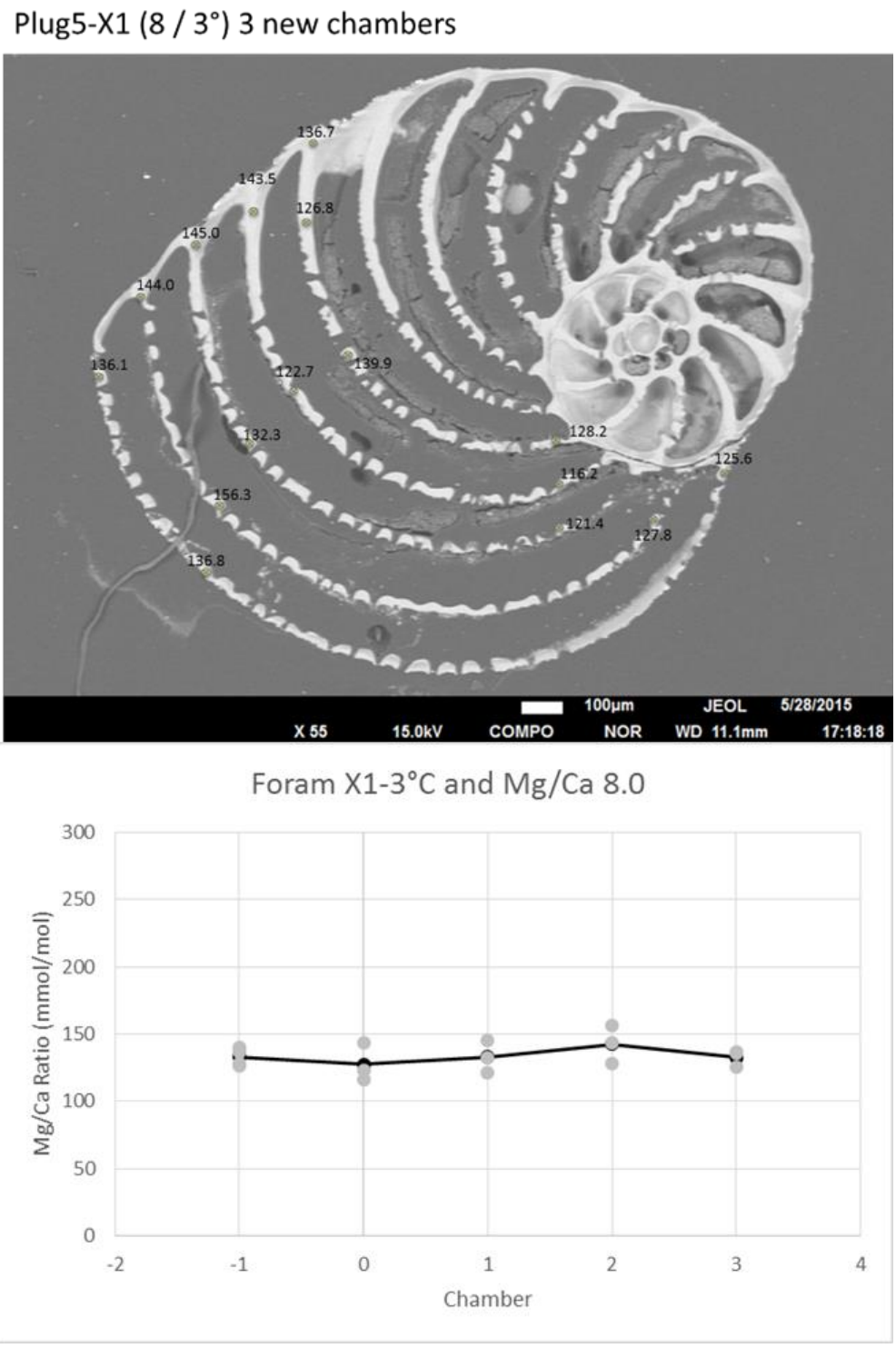


O2: SEM backscatter photomicrograph of foraminifera O2 that was placed in experimental seawater with a temperature of 3°C and a Mg/Ca ratio of 1.0. It grew 3 new chambers during the experimental process.





Z1: SEM backscatter photomicrograph of foraminifera Z1 that was placed in experimental seawater with a temperature of 3°C and a Mg/Ca ratio of 6.0. It grew 3 new chambers during the experimental process. One of those chambers (outermost one) was heavily damaged during sample processing.



X1: SEM backscatter photomicrograph of foraminifera X1 that was placed in experimental seawater with a temperature of 3°C and a Mg/Ca ratio of 8.0. It grew 3 new chambers during the experimental process.

APPENDIX D. SEAWATER RECIPE USED FOR *ELPHIDIUM* AND *PENEROPLID* DISH EXPERIMENT

	Seawater Recipe (Nominal Mg/Ca in mol/mol)				
	0.1	**1.0**	**2.5**	**5.0**	**7.0**
	Compound Weight (g)	**Compound Weight** (g)	**Compound Weight** (g)	**Compound Weight** (g)	**Compound Weight** (g)
NaCl	895.65	897.25	897.97	898.38	898.50
Na_2SO_4	150.04	150.30	150.42	150.49	150.51
KCl	25.34	25.39	25.41	25.42	25.42
$NaHCO_3$	7.34	7.35	7.36	7.36	7.36
KBr	3.67	3.68	3.68	3.68	3.68
H_3BO_3	0.97	0.98	0.98	0.98	0.98
NaF	0.11	0.11	0.11	0.11	0.11
$SrCl_2•6H_2O$	0.90	0.90	0.90	0.90	0.90
$MgCl_2•6H_2O$	43.74	241.00	344.56	404.77	422.34
$CaCl_2•2H_2O$	306.33	174.28	99.66	56.29	43.63
H_2O	36000.00	36000.00	36000.00	36000.00	36000.00
Mg (mol/kg)	0.01	0.03	0.05	0.05	0.06
Ca (mol/kg)	0.06	0.03	0.02	0.01	0.01
Actual Mg/Ca (mol/mol)	**0.10**	**0.97**	**2.42**	**5.04**	**6.78**

Methods

Total moles of magnesium plus calcium were kept constant for each batch of artificial seawater. Seawater was mixed in batches of 3L in a multistep process. First, gravimetric salts (salts that are not hydrated) were dried at 150^{o}C for approximately two weeks prior to mixing. All ingredients except hydrated salts were then weighed and added to 2L of water. Hydrated salts were weighed and added to the amount of remaining water, in a separate container. Hydrated salt solution was added to the gravimetric salt solution and mixed. Salinity was 30ppt for every mixture.

APPENDIX E. RESULTS *ELPHIDIUM* EXPERIMENT

		Mg/Ca (mol/mol)				
		0.1	**1.0**	**2.5**	**5.0**	**7.0**
Temperature	**2°C**	ARAG	X	X	X	DISSOLVED
	8°C	ARAG	X	X	X	DISSOLVED
	16°C	ARAG	X	X	X	DISSOLVED
	25°C	ARAG	X	X	X	DISSOLVED

Seawater Mg/Ca and Temperature conditions for *Elphidium* dish experiment, "ARAG" indicates aragonite precipitated in the dish, "X" indicates that no growth occurred during the experiment, "DISSOLVED" indicates that the foraminifera dissolved either during the experiment, or at the end of the experiment during euthanasia.

Methods

The table above summarized an experiment done on *Elphidium excavatum*. This foraminifera is a low-Mg calcite, benthic foraminifera. It is approximately 75 microns across on average. *E. excavatum* were collected in Long Island Sound, CT at 41°15'3.78"N and 72°43'31.31"W in approximately 20 meters of water.

Seawater was mixed according to the recipe in Appendix D. Twenty five petri dishes of foraminifera were picked, each dish corresponding to a specific temperature and seawater Mg/Ca. Approximately 75 *Elphidium* were placed in each dish, which were then covered with a lid. Subsequently, each dish was placed in an incubator set to the experimental temperature.

Calcein was added to the water at a concentration of 100mg/L for the first three weeks of the experiment. Foraminifera were then left to grow for approximately 37 days after being put in water without calcein. Foraminifera grew for a total of 58 days.

During this study, seawater was changed every three days. This was done with a clean syringe after foraminifera were swirled to the center of the dish. Foraminifera were fed a diatom mixture once per week. This mixture was purchased from Carolina Biological. Diatoms overgrew in all dishes that were kept at 25°C. During water changes, as many diatoms were removed as possible.

Results

After the 58-day period, the experiment was concluded. Foraminifera were removed, placed in ethanol for one day and then transferred to distilled water. After foraminifera were transferred to water, they were observed within one day. The table in this appendix displays the results of the study. Boxes with "CALCITE" signify that calcite precipitated in the petri dish and was therefore unusable, as water chemistry had been altered. Boxes with "X" signify that no growth occurred, though foraminifera appeared to still be alive at the end of the 58-day period.

Boxes marked with “DISSOLVED” signify that foraminifera in those dishes dissolved; this is likely due to the low levels of calcium in the seawater. Overall, no foraminifera were usable from this experiment.

APPENDIX F. RESULTS OF *PENEROPLID* EXPERIMENT

		Mg/Ca (mol/mol)				
		0.1	**1**	**2.5**	**5**	**7**
Temperature	**25°C**	ARAG	X	X	12 NEW	14 NEW

Methods

The table above summarized an experiment done on *Peneroplis planatus*. This foraminifera is a high-Mg calcite, benthic foraminifera. It is approximately 200 microns across on average. *P. planatus* were collected in Al Thakira, Qatar at 25° 45' 7.01" N and 51° 33' 50.12" E in a shallow intertidal zone.

Seawater was mixed according to the recipe in Appendix D. Five petri dishes of foraminifera were picked, each dish corresponding to a specific seawater Mg/Ca. All dishes were kept at 25ºC. Each dish contained approximately five peneroplids. After foraminifera were placed in dishes, each dish was covered with a lid and placed in the 25ºC refrigerator.

Foraminifera were left to grow for 28 days. Calcein was added to the water at a concentration of 100mg/L for the first week of the experiment. Foraminifera were then left to grow for approximately 21 days after being put in water without calcein.

During this study, seawater was changed every three days. Foraminifera were fed a diatom mixture every other week. This mixture was purchased from Carolina Biological. During water changes, as many diatoms were removed as possible.

Results

After the 28-day period, the experiment was concluded. Foraminifera were removed, placed in ethanol for one day and then transferred to a microcentrifuge vial where they desiccated. After foraminifera were transferred to vials, they were observed within one day. The table in this appendix displays the results of the study. Boxes with “ARAG” signify that aragonite precipitated in the petri dish and was therefore unusable, as water chemistry had been altered. Boxes with “X” signify that no growth occurred, though foraminifera appeared to still be alive at the end of the 28 day period. Boxes marked with “# NEW” signify that three foraminifera in those dishes grew approximately “#” of new chambers. All new chambers grown were malformed.